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KINETIC ANALYSIS OF A SERIES OF PHOSPHOTRIESTERASE MUTANTS WITH RESPECT TO SARIN AND SOMAN CATALYSIS

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13 ABSTRACT *(Maximum 200 words)*

A series of site-directed mutants of the organophosphorus-hydrolyzing enzyme phosphotriesterase (PTE) were analyzed with respect to their kinetics on the G-agent substrates sarin (GB) and soman (GD). Values of Km, Vmax, and Kcat/Km were compared with the wild-type values. Several mutants were found with decreased Km values on both substrates, although all had at least somewhat decreased Vmax values as well. The Kcat/Km calculations showed two mutants (G60A and H254G/H257R) with GB catalytic efficiencies nearly as high as wild-type. With respect to GD catalytic efficiency, both these mutants were in the same Kcat/Km range or higher than wild-type. In addition to their potential practical utility for decontamination, these results also provide part of the roadmap for future mutagenesis studies with the PTE enzyme.

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PREFACE

The work described in this report was authorized under Project No. 100600, Tech Base Program. This work was started in October 2000 and completed in July 2002.

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KINETIC ANALYSIS OF A SERIES OF PHOSPHOTRIESTERASE MUTANTS WITH RESPECT TO SARIN AND SOMAN CATALYSIS

1. INTRODUCTION

The phosphotriesterase (PTE) enzyme catalyzes the hydrolysis of a number of different pesticides and chemical nerve agents. As a catalytic decontaminant of chemical nerve agents, PTE has several attractive features. The gene for PTE has been cloned into *Escherichia coli* and active enzyme can be produced in quantity.[1] Also, knowledge of the structure of the PTE active site permits rational design of mutants for the purpose of increasing activity or broadening the substrate range.[2] For instance, site-directed mutagenesis studies have previously identified PTE mutants with enhanced soman[3] and VX[4, 5] catalysis.

While some G-agent work has been completed on kinetics of the wild-type enzyme and the initial rate kinetics of some of the mutants, mutant enzymes are much less well characterized with respect to agent kinetics. Detailed kinetic data are important to determine appropriate potential applications for enzyme variants as well as to provide a roadmap for future mutagenesis studies.

2. MATERIALS AND METHODS

2.1. Enzyme Assays.

Enzyme assays were conducted with a fluoride electrode attached to a Fisher Accumet 925 meter. Reactions were conducted in a temperature-controlled vessel in a total volume of 5 mL. Buffering was provided by a 50 mM solution of bis-tris-propane at pH 7.2.

Kinetic parameters were determined from assays conducted at 25 °C. EZ-FIT® version 5.03 software[6] was used for determination of kinetic parameters such as Km and Vmax, and the accompanying statistical analyses. EZ-Fit uses the Nelder-Mead simplex and Marquardt nonlinear regression algorithms sequentially to determine the kinetic parameters from the Michaelis-Menton plot of velocity versus substrate concentration. EZ-FIT® uses two regression methods in tandem to fit the Km, Vmax, and inhibition data to a non-linear curve. The uncertainty values on the Kcat/Km calculations were determined by adding the percent uncertainty of the Kcat value and the percent uncertainty of the Km value and multiplying by the calculated Kcat/Km value then dividing by 100.

2.2. Construction of Mutant PTE Strains.

Site-directed mutants were constructed using the method of overlap extension and cassette insertion with a synthetic gene (Wohlschlegel, personal communication).

3. RESULTS AND DISCUSSION

Assays were run with varying substrate concentrations of sarin (GB) and soman (GD) in order to produce substrate saturation plots (specific activity versus substrate concentration). Kinetic data are summarized in the figures below; raw data, detailed kinetic plots, and summary tables are provided in the appendices that follow.

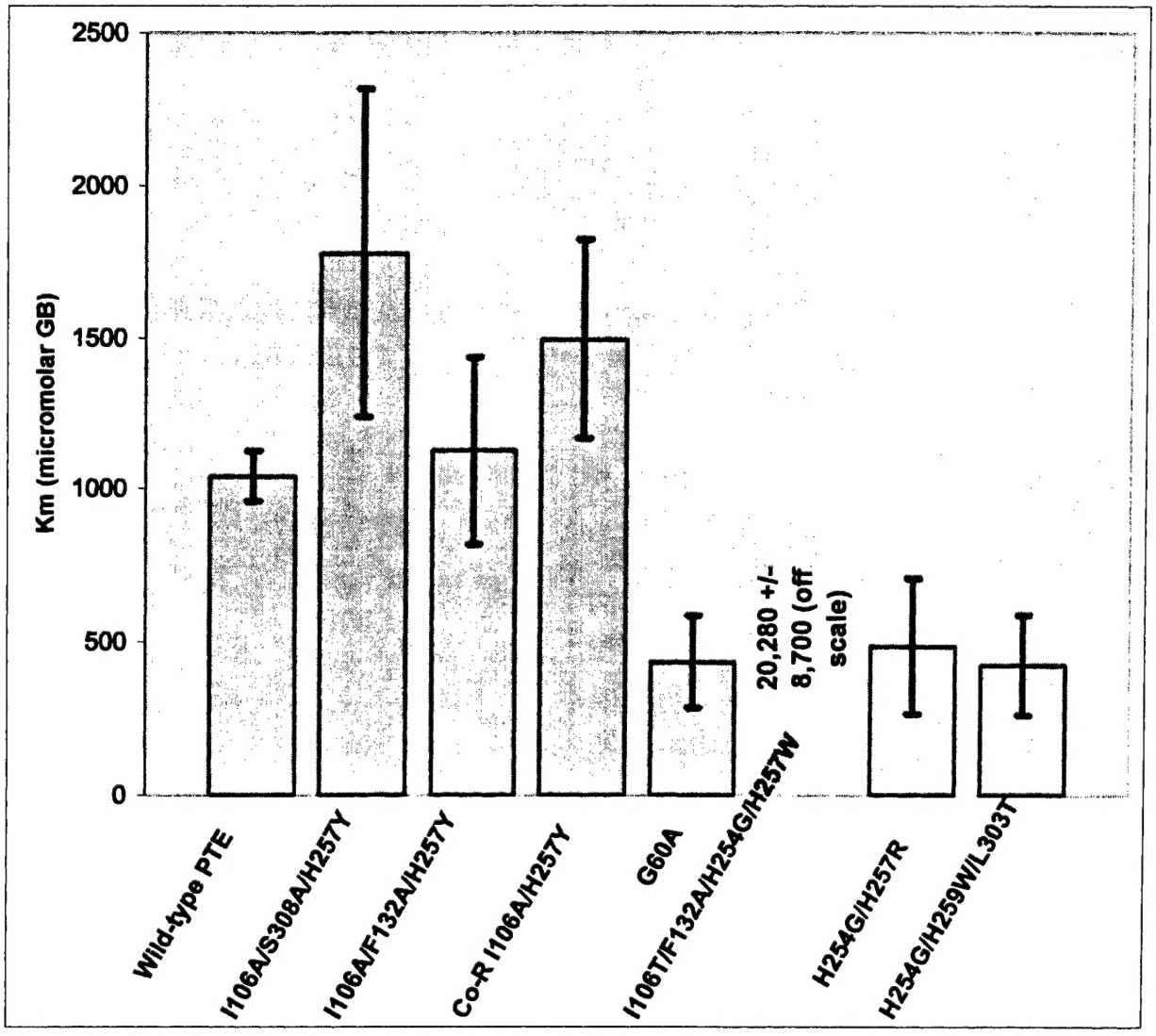

Figure 1. Km values of wild-type PTE and seven mutants with GB as the substrate.

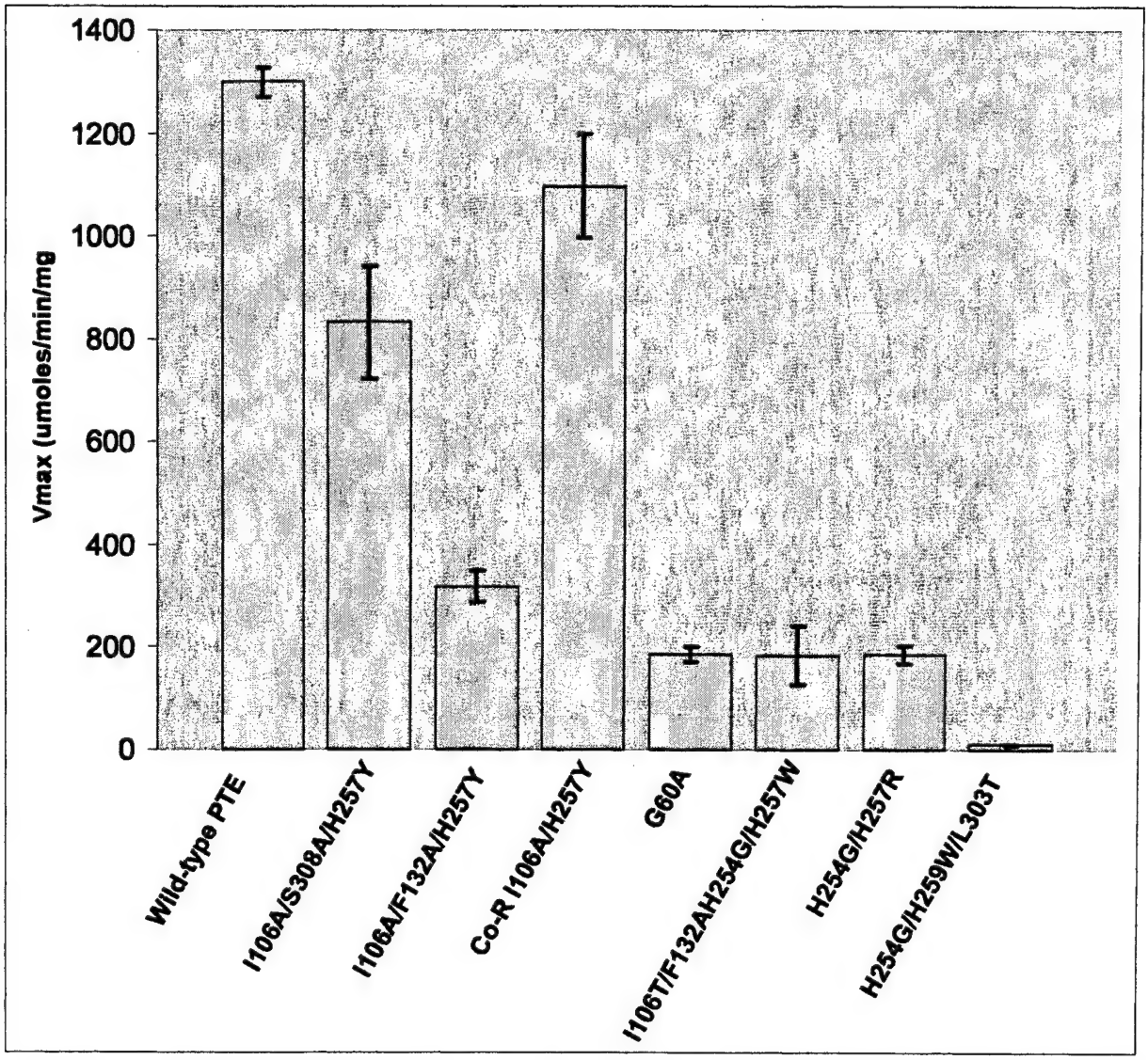

Figure 2. Vmax values of wild-type PTE and seven mutants with GB as the substrate.

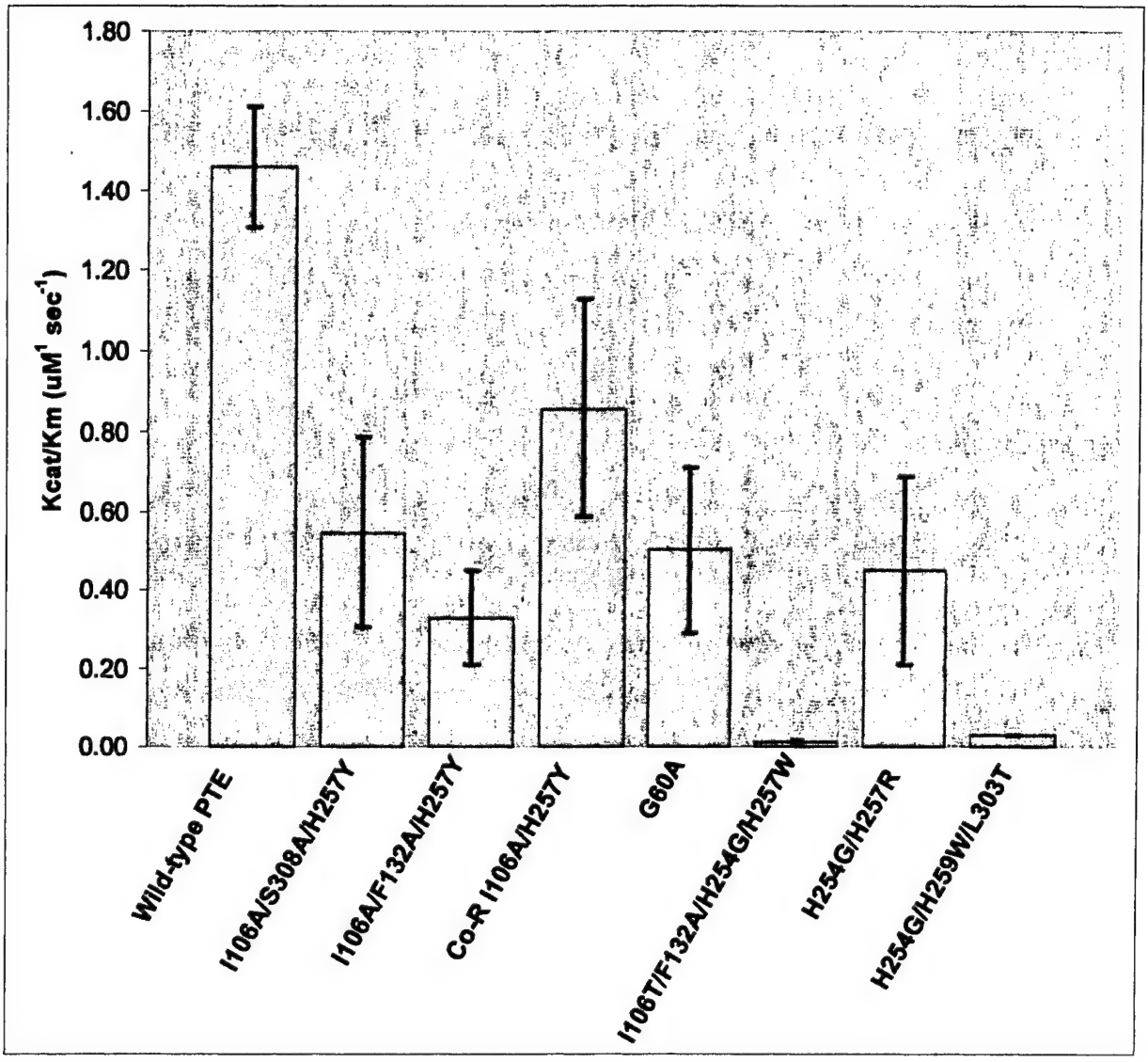

Figure 3. Kcat/Km values of wild-type PTE and seven mutants with GB as the substrate.

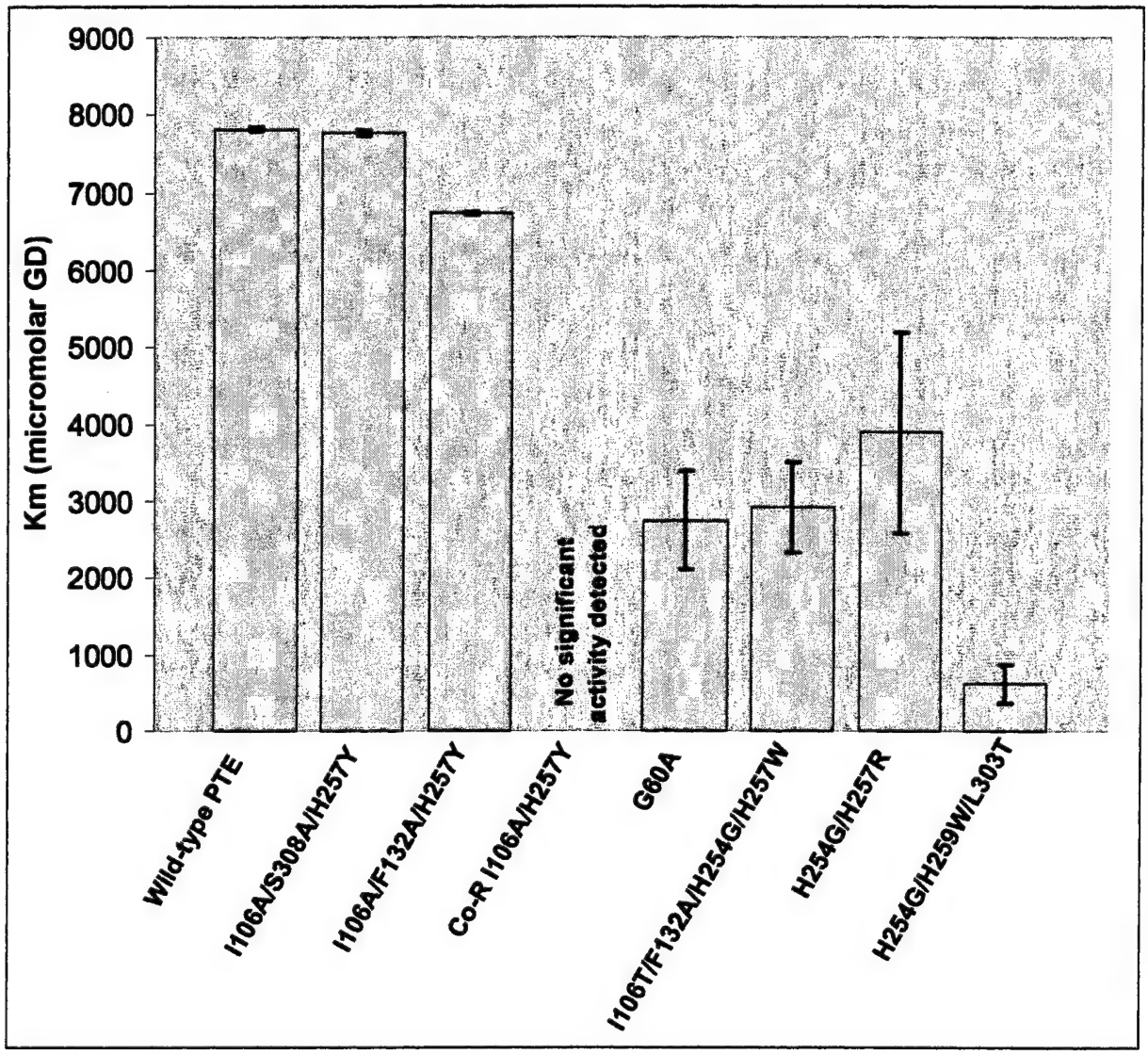

Figure 4. Km values of wild-type PTE and seven mutants with GD as the substrate.

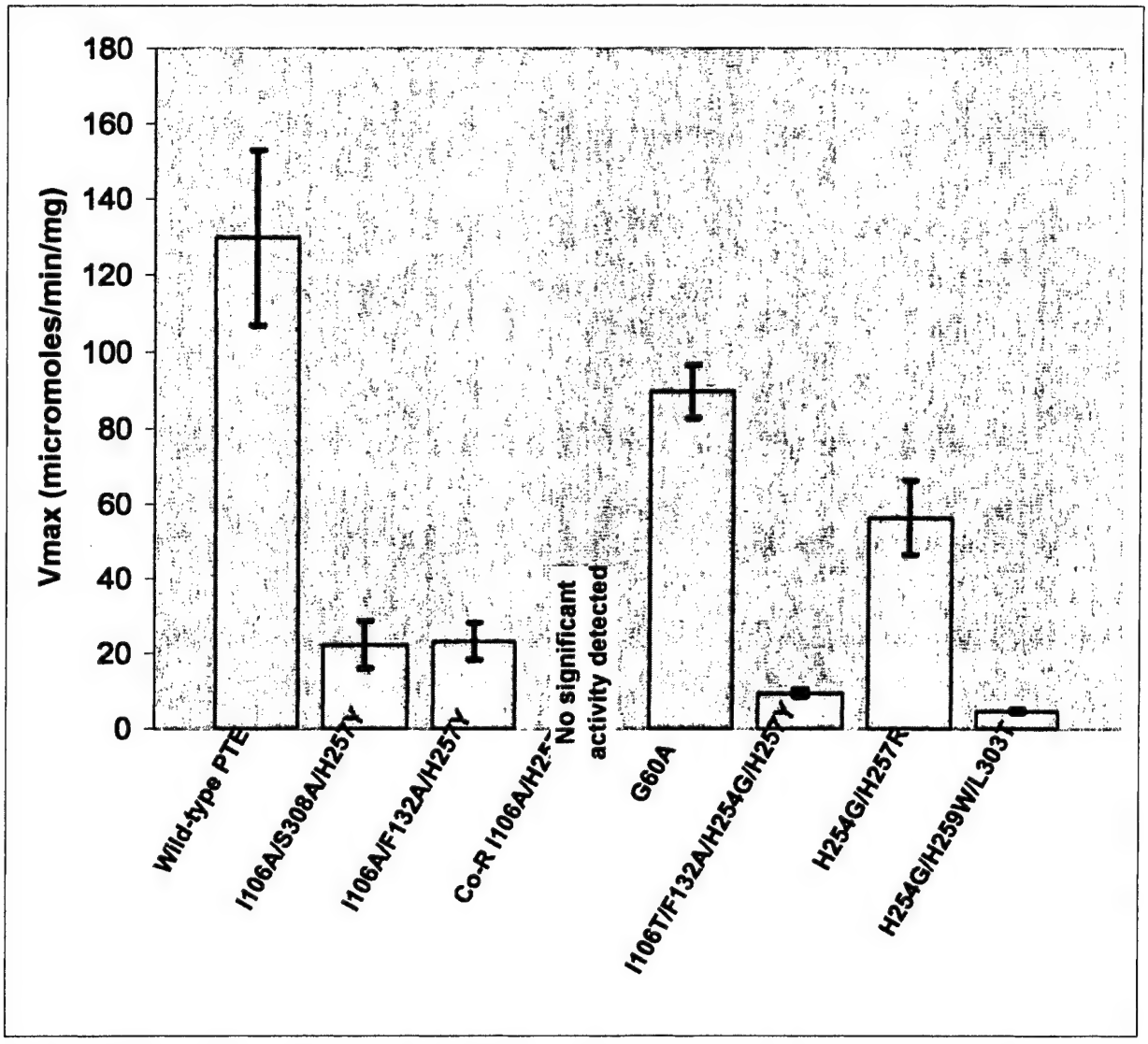

Figure 5. Vmax values of wild-type PTE and seven mutants with GD as the substrate.

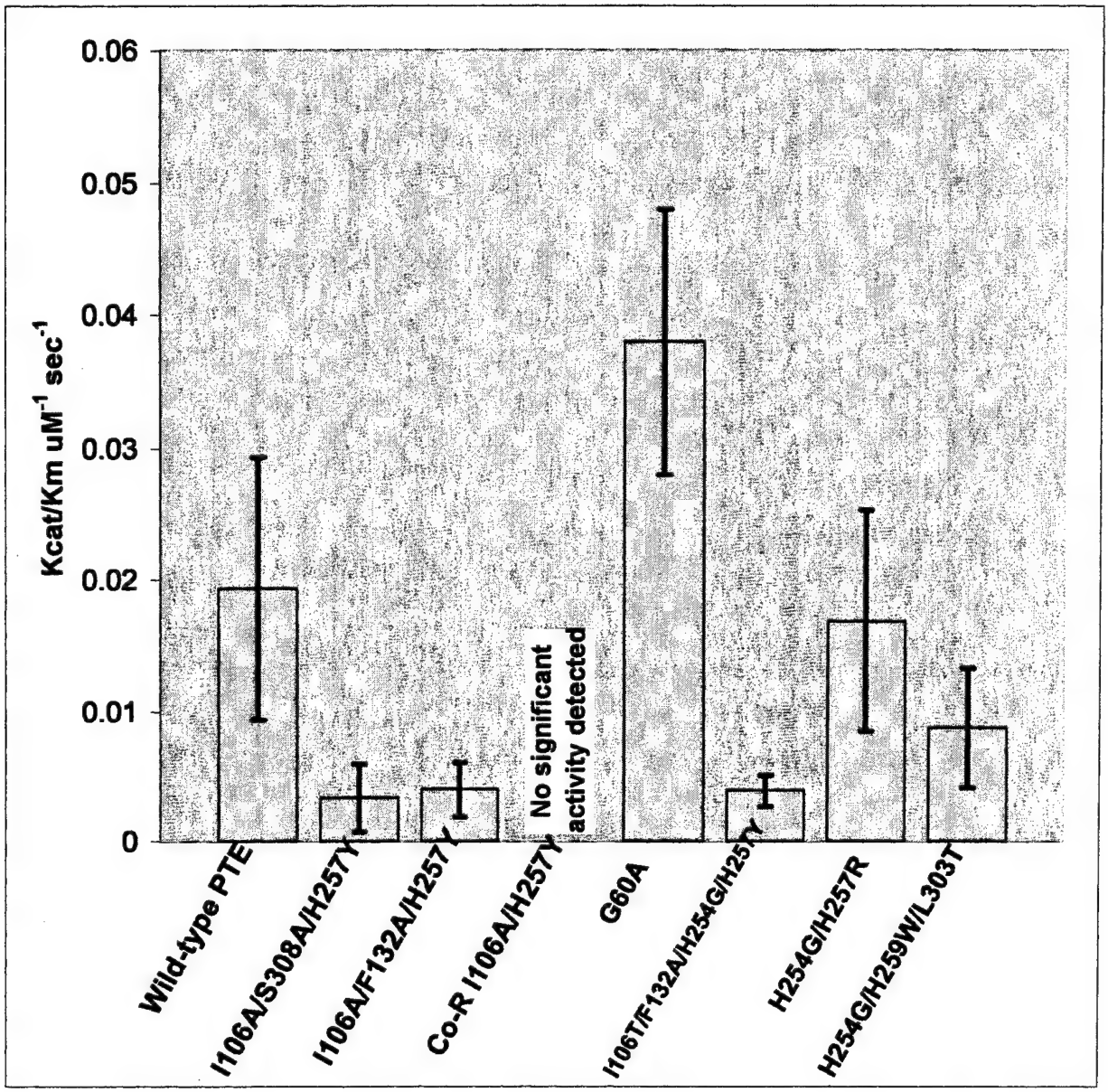

Figure 6. Kcat/Km values of wild-type PTE and seven mutants with GD as the substrate.

4. CONCLUSIONS

With respect to Km values (substrate concentration at half maximal velocity), smaller numbers generally indicate more useful enzymes, since these enzymes are able to function more efficiently with lower substrate concentrations. For Vmax values (maximal velocity at a saturating substrate concentration), higher numbers are generally sought for decontamination purposes, since these enzymes will provide faster decontamination rates. Kcat values are a function of the Vmax and the molecular weight of the enzyme and Kcat/Km (specificity constant) calculations provide a means of evaluating the enzymes' catalytic efficiency while incorporating both the Km and the rate. With this value, larger numbers equate with greater catalytic efficiency.

For the GB experiments, three mutants (G60A, H254G/H257R, and H254G/H259W/L303T) exhibited decreased Km values as compared to the wild-type PTE enzyme. The Km values for all three were approximately half that of wild-type. One mutant (I106T/F132A/H254G/H257W) exhibited a very large Km value, approximately 20 times that of the wild-type enzyme. The Km values for the other three mutants were either in the same range as wild-type or slightly higher.

GB Vmax values however, were all decreased as compared to the wild-type enzyme. Consequently, the Kcat/Km values were all decreased as compared to wild-type. However, two of the three mutants with lowered Km values (G60A and H254G/H257R) retain Kcat/Km values about half that of the wild-type enzyme. As such, they still represent potentially useful mutants.

For the GD experiments, there were also several mutants with significantly decreased Km values. G60A, I106T/F132A/H254G/H257W, and H254G/H257R all had Km values about half that of the wild-type enzyme (which itself, was about eight times higher than the same enzyme with GB). Also, H254G/H259W/L303T had an extremely low Km, less than 1/12 that of the wild-type enzyme. Of the mutants with lower Km values, two (G60A and H254GH257R) had Vmax values at least approximately half that of wild-type (which itself was about 1/10 as high as with GB). Kcat/Km calculations revealed that the G60A mutant generally had a greater catalytic efficiency than wild-type, although the error bars overlapped slightly. H254G/H257R, with its decreased Km value, has a Kcat/Km value significantly similar to wild-type.

In conclusion, with respect to their kinetics on GB and GD, the two most interesting mutants of this series were G60A and H254G/H257R. Both mutants had decreased Km values with both substrates and while Vmax values were decreased in all cases, the Kcat/Km values ranged between 1/2 and twice that of the wild-type enzyme.

LITERATURE CITED

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6. Perrella, F.W.; "EZ-FIT: A Practical Curve-Fitting Program for the Analysis of Enzyme Kinetic Data on IBM-PC Compatible Computers," Analytical Biochemistry Vol. 174, pp 437-447 (1988).

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APPENDIX

RAW KINETIC DATA

A. Raw Data: Wild-type PTE on GB

==========

Date: Monday November 19, 2001 2:06 pm
Filename: C:\MYDOCU~1\EZFITD~1\2001WTGB.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -2.47 Akaike Criterion= 61.476
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0478

Km:= 1040.41 +/- 84.4426 (p=3.118E-05)
Vmax:= 1299.889 +/- 28.3205 (p=4.645E-08)

Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.857	1.000

==========

Velocity, Dose, Inhibitor
404.153,480,0
641.014,960,0
785.5,1800,0
976.806,3000,0
1074.17,4200,0
1115,6000,0
1139.72,9000,0
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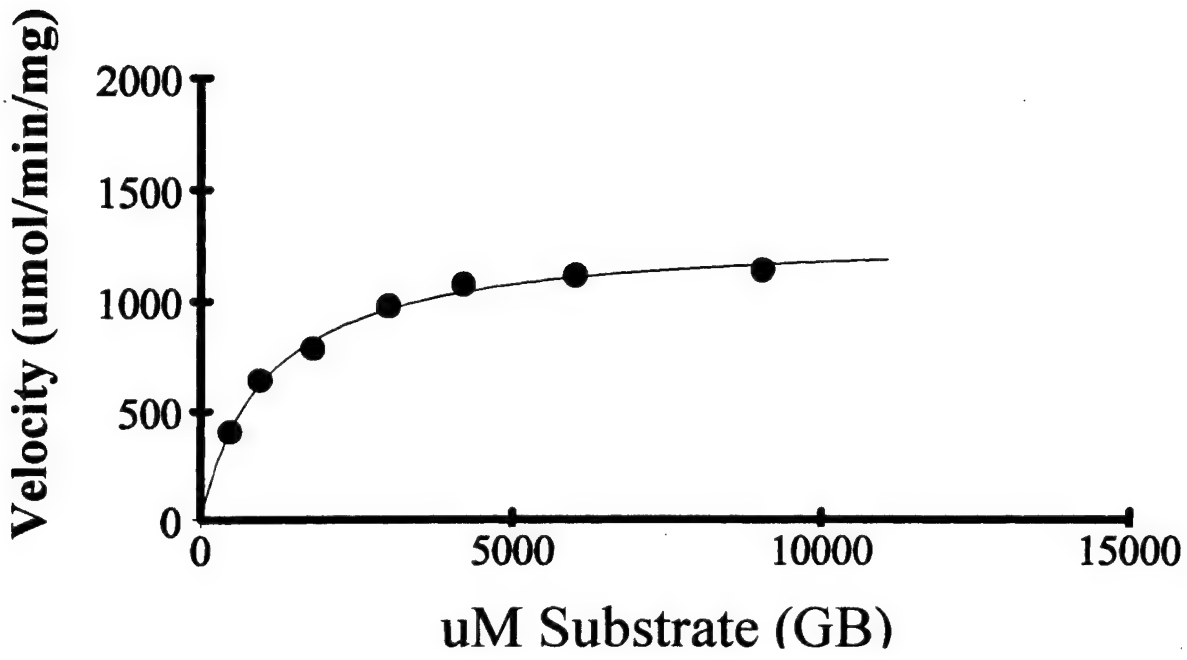
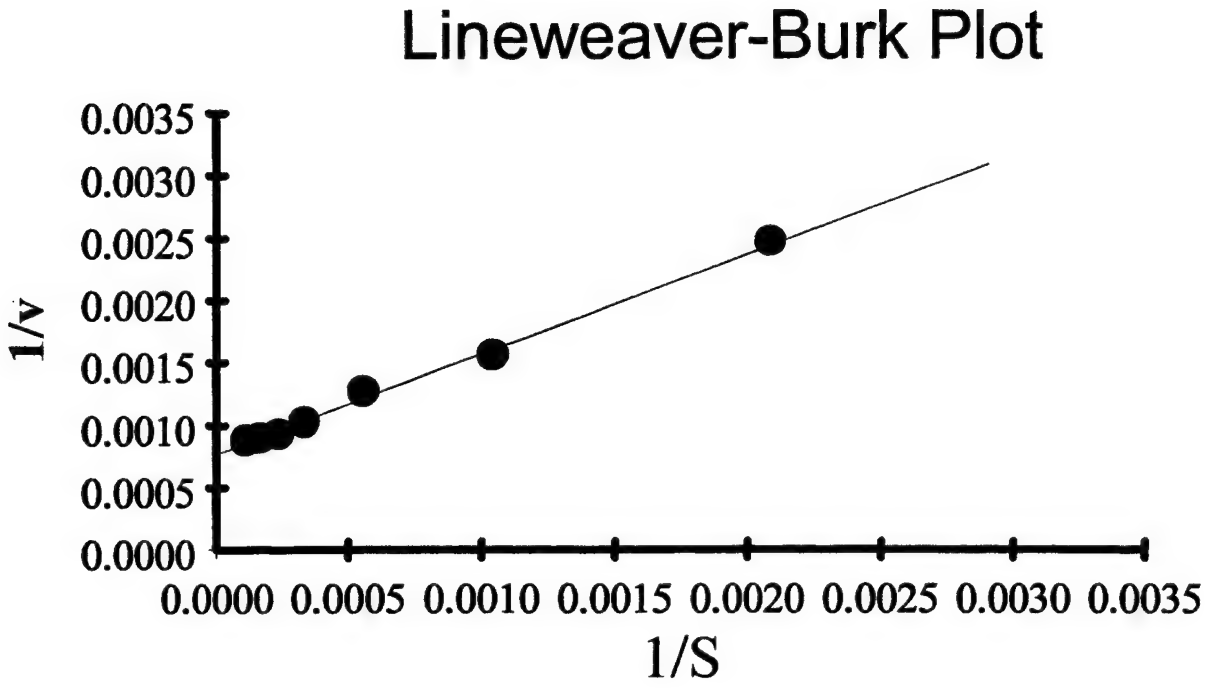
Substrate Saturation Plot: Wild-type PTE on GB



Lineweaver-Burk Plot: Wild-type PTE on GB



B. Raw Data: I106A/S308A/H257Y PTE on GB

Begin Results: C:\EZFIT5\ISH-GB.RAW

==========

Date: Tuesday February 26, 2002 12:25 pm
Filename:
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -.618 Akaike Criterion= 78.448
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .3271

Km:= 1777.79 +/- 540.9551 (p=8.344E-03)
Vmax:= 833.1934 +/- 105.8878 (p=1.115E-04)

Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.962	1.000

==========

Velocity, Dose, Inhibitor
98.5426,300,0
155.62,600,0
332.326,1200,0
471.337,1800,0
503.45,2400,0
548.76,3000,0
562.868,3600,0
526.085,4200,0
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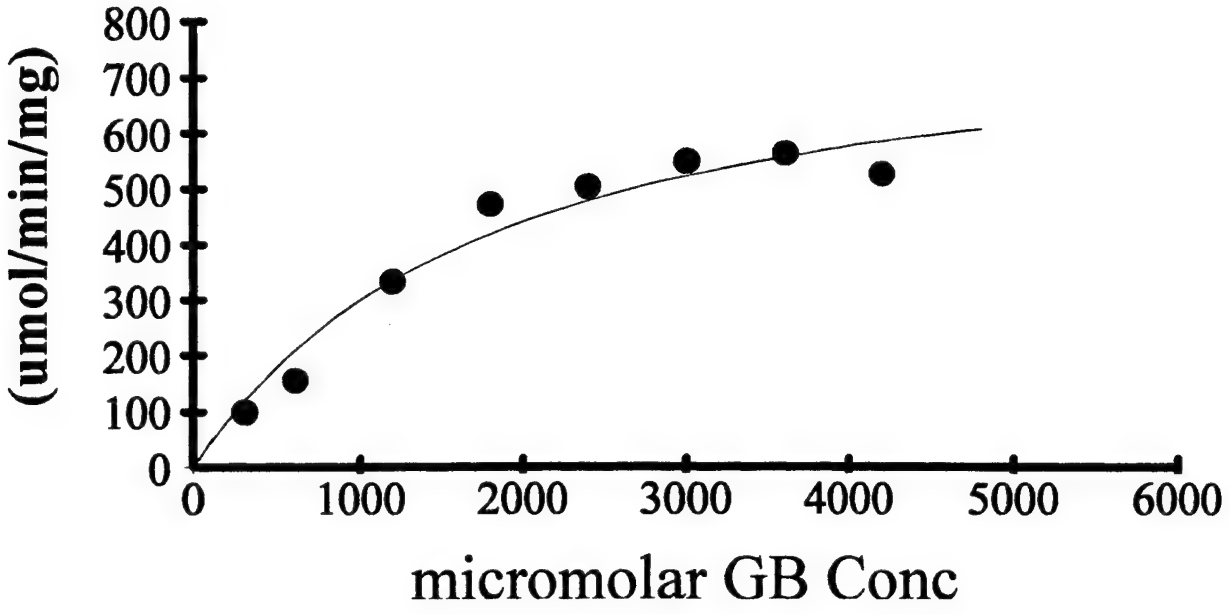
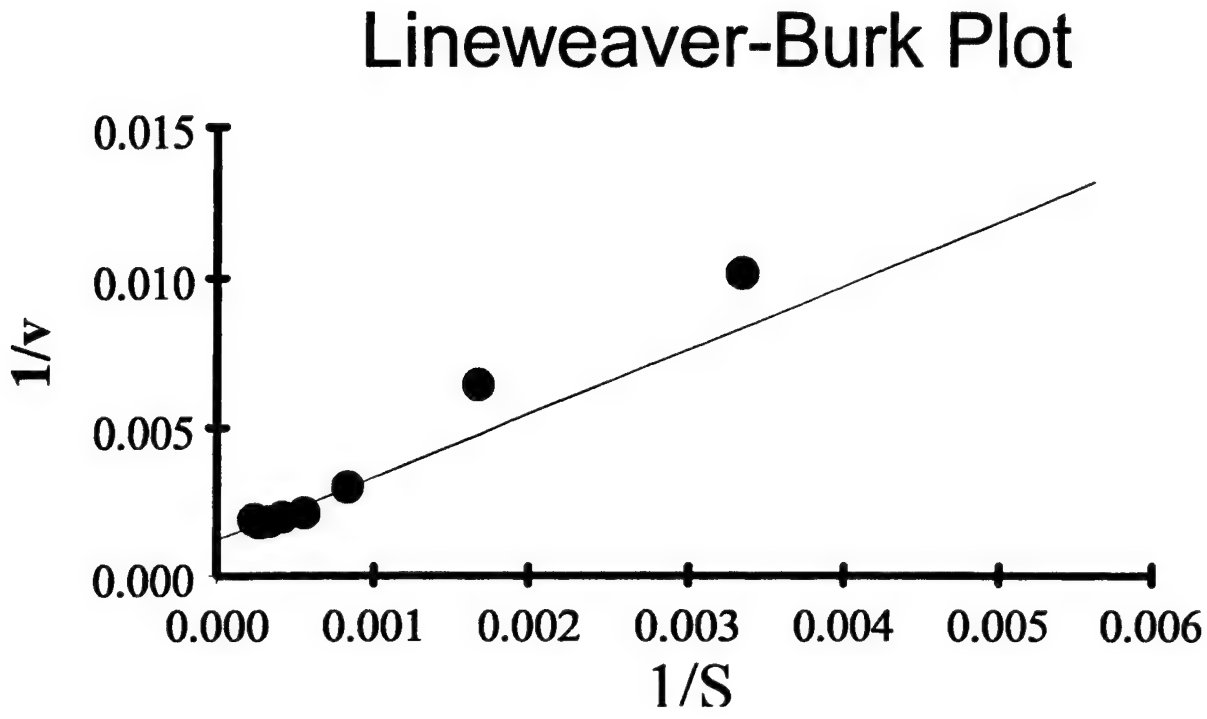
Substrate Saturation Plot: I106A/S308A/H257Y PTE on GB



Lineweaver-Burk Plot: I106A/S308A/H257Y PTE on GB



C. Raw Data: I106A/F132AH257Y on GB

==========

Date: Wednesday February 27, 2002 8:55 am
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Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -.337 Akaike Criterion= 64.982
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .4329

Km:= 1124.735 +/- 309.794 (p=5.479E-03)
Vmax:= 318.8191 +/- 29.723 (p=1.944E-05)

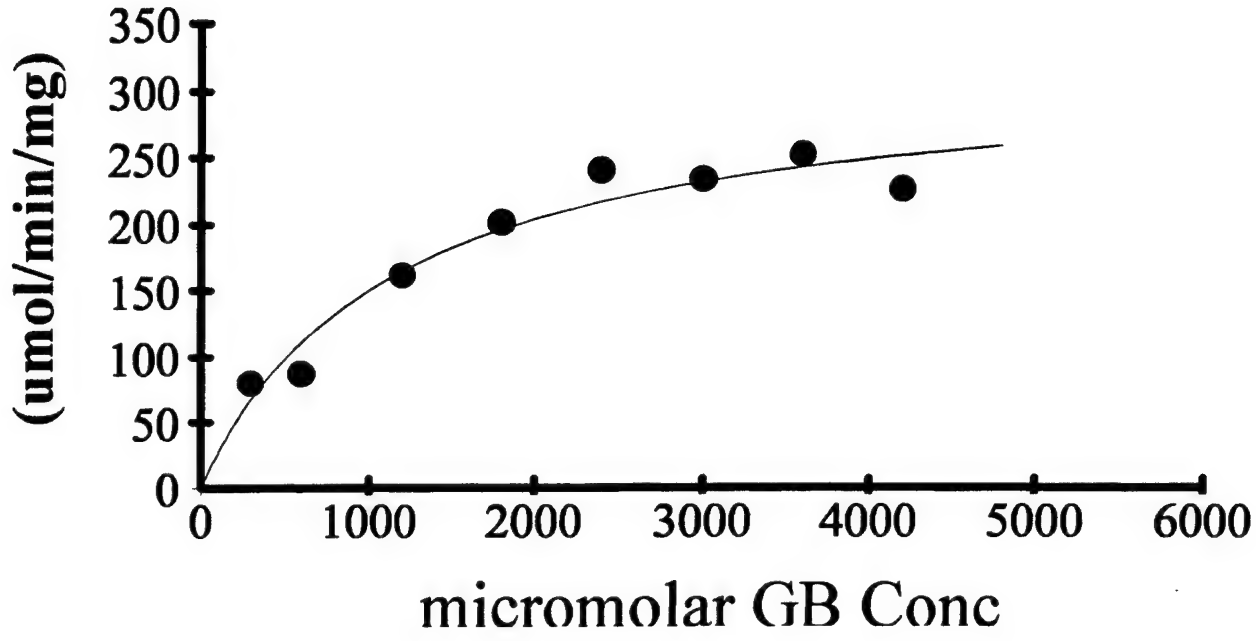
Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.934	1.000

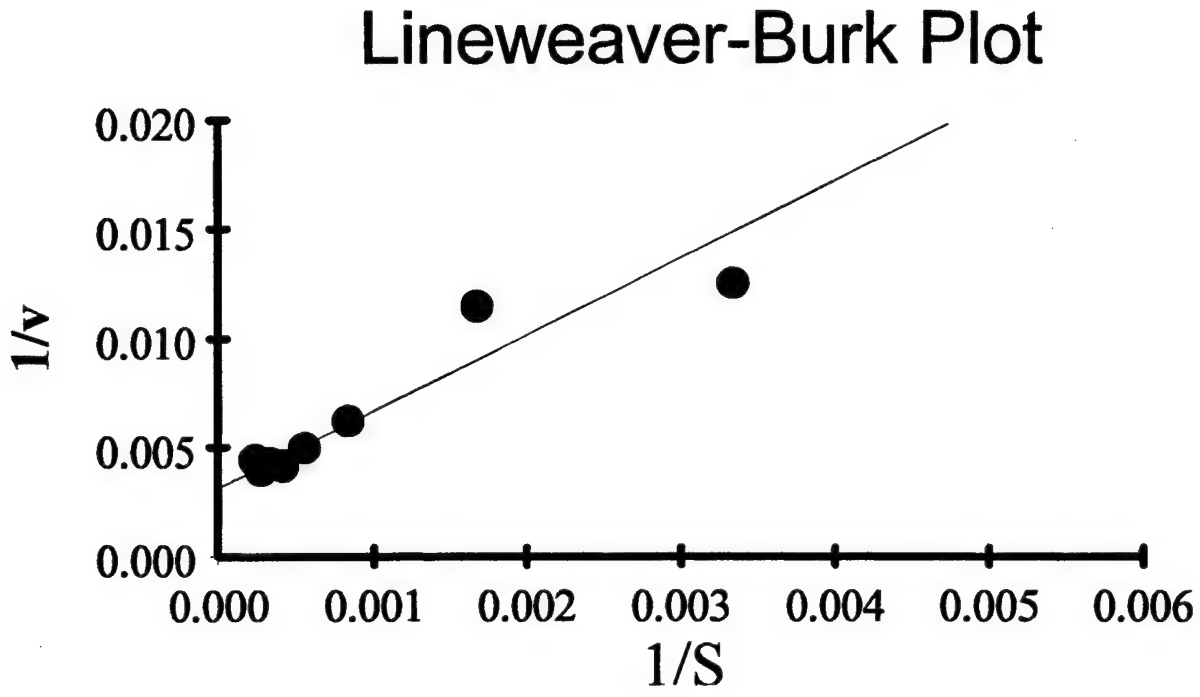
==========

Velocity, Dose, Inhibitor
79.9232,300,0
87.1094,600,0
161.523,1200,0
201.315,1800,0
240.456,2400,0
233.581,3000,0
252.018,3600,0
226.042,4200,0
End Results: C:\EZFIT5\IFH-GB.RAW

Saturation Plot: I106A/F132AH257Y on GB



Lineweaver-Burk Plot: : I106A/F132AH257Y on GB



D. Raw Data: Co-R I106A/H257Y on GB

Begin Results: C:\EZFIT5\COR-IHGB.RAW

==========

Date: Thursday February 28, 2002 7:56 pm
Filename: C:\EZFIT5\COR-IHGB.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -.087 Akaike Criterion= 125.158
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .6567

Km:= 1494.125 +/- 331.8823 (p=5.698E-04)
Vmax:= 1097.99 +/- 100.745 (p=3.716E-07)

Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.966	1.000

==========

Velocity, Dose, Inhibitor
191.341,300,0
301.929,600,0
432.717,900,0
469.244,1200,0
558.106,1500,0
629.734,1800,0
660.003,2100,0
648.411,2400,0
602.189,2700,0
751.508,3000,0
850.762,3300,0
746.968,3600,0
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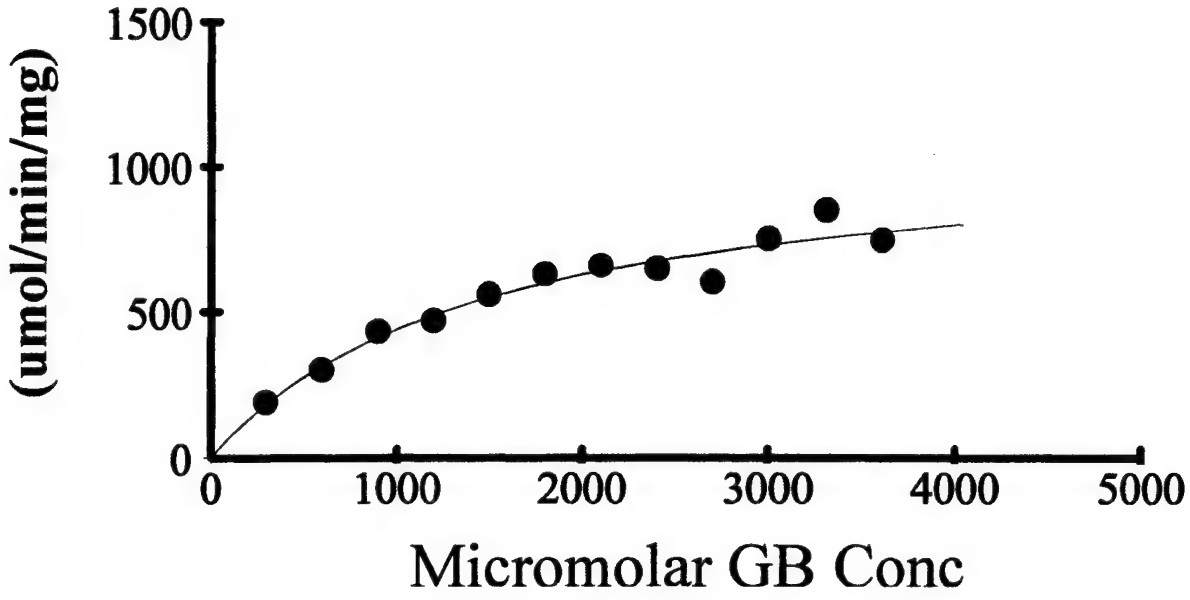
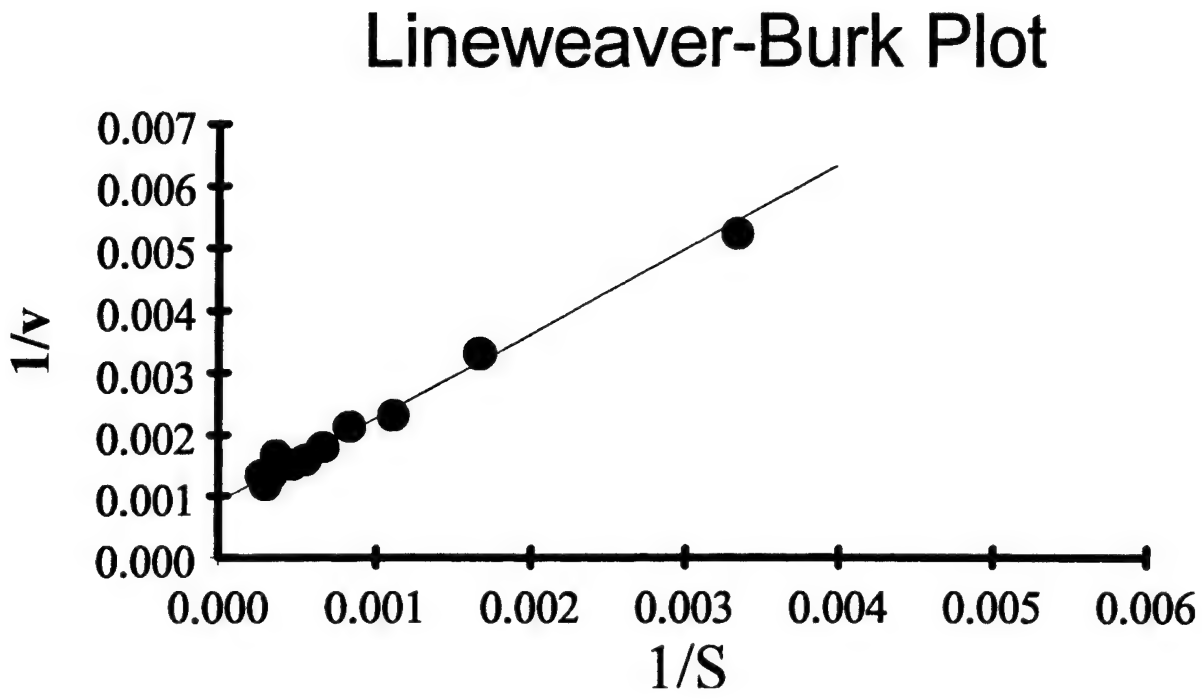
Saturation Plot: Co-R I106A/H257Y on GB



Lineweaver-Burk Plot: Co-R I106A/H257Y on GB



E. Raw Data: G60A on GB

Begin Results: C:\EZFIT5\G60-GB.RAW

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Filename: C:\EZFIT5\G60-GB.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= .262 Akaike Criterion= 55.675
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .734

Km:= 433.3417 +/- 148.0088 (p=1.636E-02)
Vmax:= 186.9401 +/- 14.6026 (p=2.588E-05)

Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.788	1.000

==========

Velocity, Dose, Inhibitor
48.8264,240,0
95.3030,480,0
138.5269,960,0
174.9369,1800,0
165.4461,3000,0
172.0539,4200,0
150.1263,6000,0
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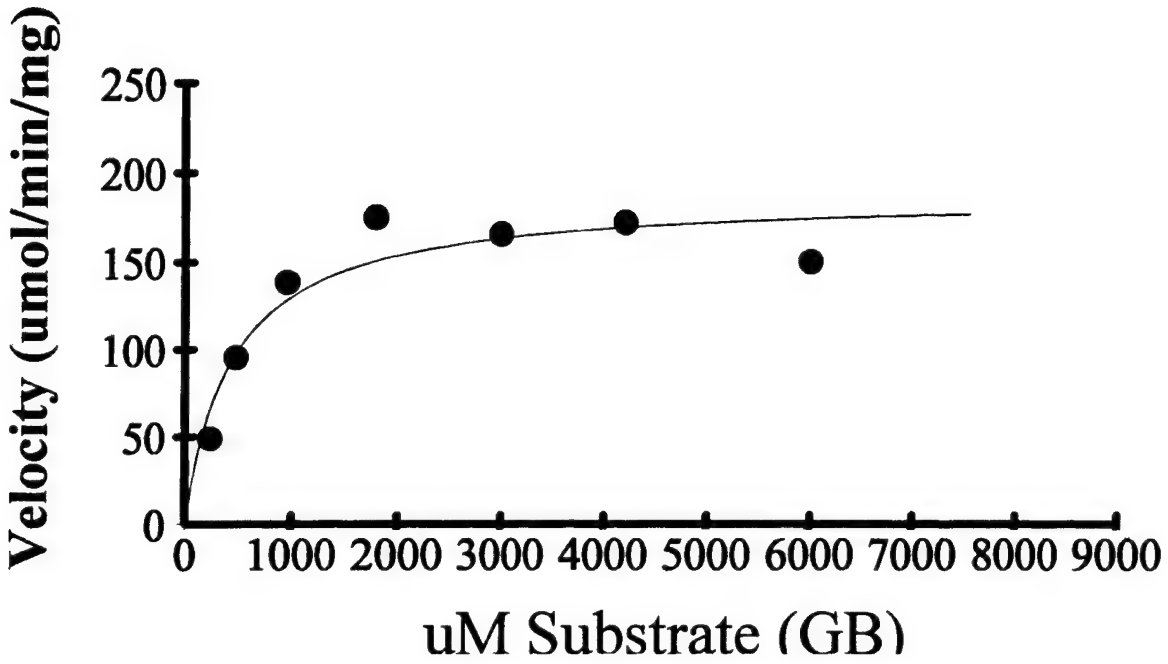
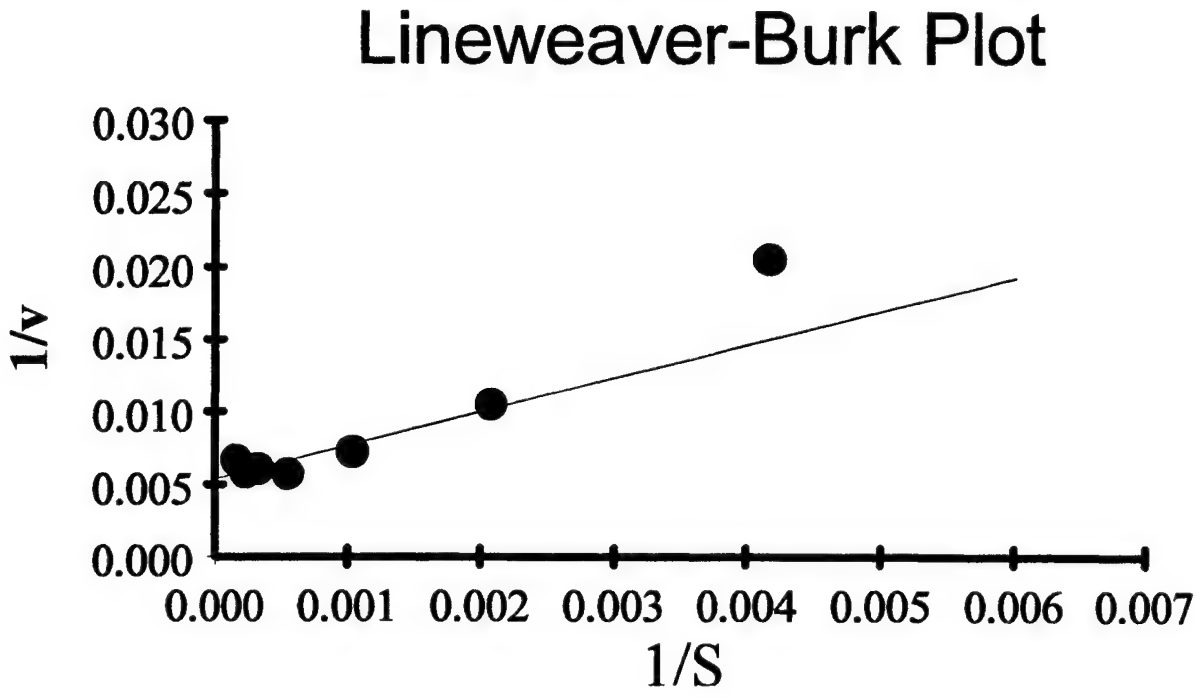
Saturation Plot: G60A on GB



Lineweaver-Burk Plot: G60A on GB



F. Raw Data: I106T/F132A/H254G/H257W on GB

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Date: Friday April 19, 2002 3:49 pm
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Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= .378 Akaike Criterion= 119.763
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= 1.1684

Km:= 20277.2 +/- 8695.819 (p=1.655E-02)
Vmax:= 185.2358 +/- 55.7377 (p=2.151E-03)

Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.993	1.000

==========

Velocity, Dose, Inhibitor
5.1946,300,0
7.5637,600,0
9.2274,900,0
16.7131,1200,0
16.46786,1800,0
19.7631,2400,0
18.83571,3000,0
26.16071,3600,0
22.0119,4200,0
27.85714,4800,0
34.89881,5400,0
53.68452,6000,0
53.27976,7200,0
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55.15476,9600,0
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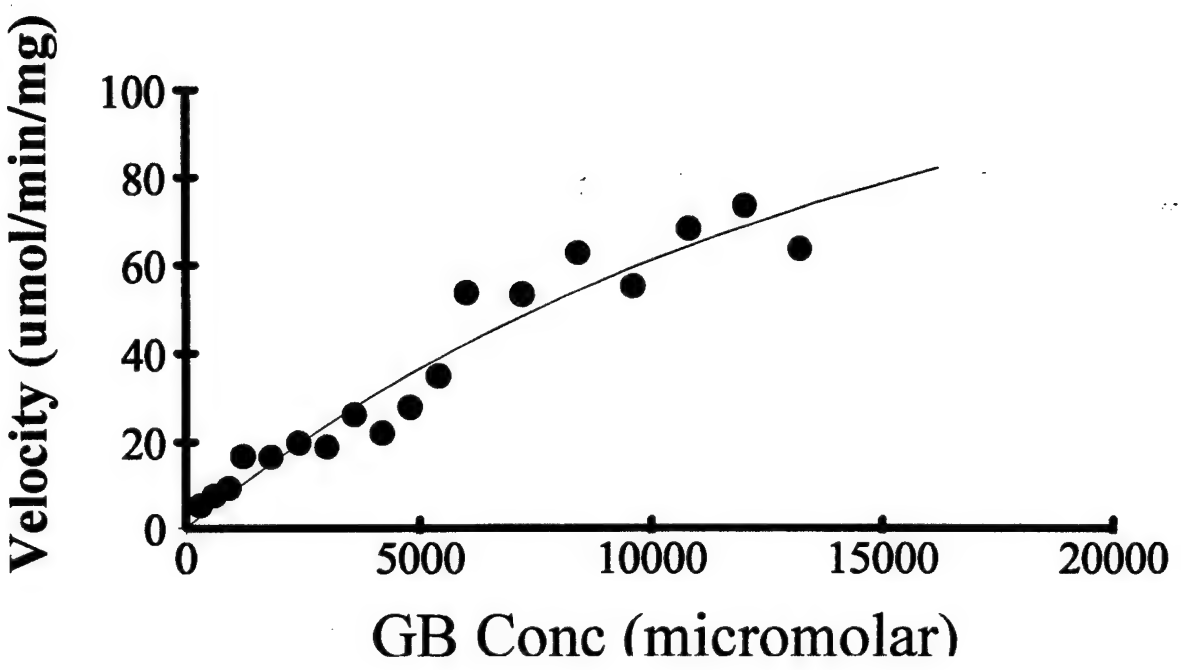
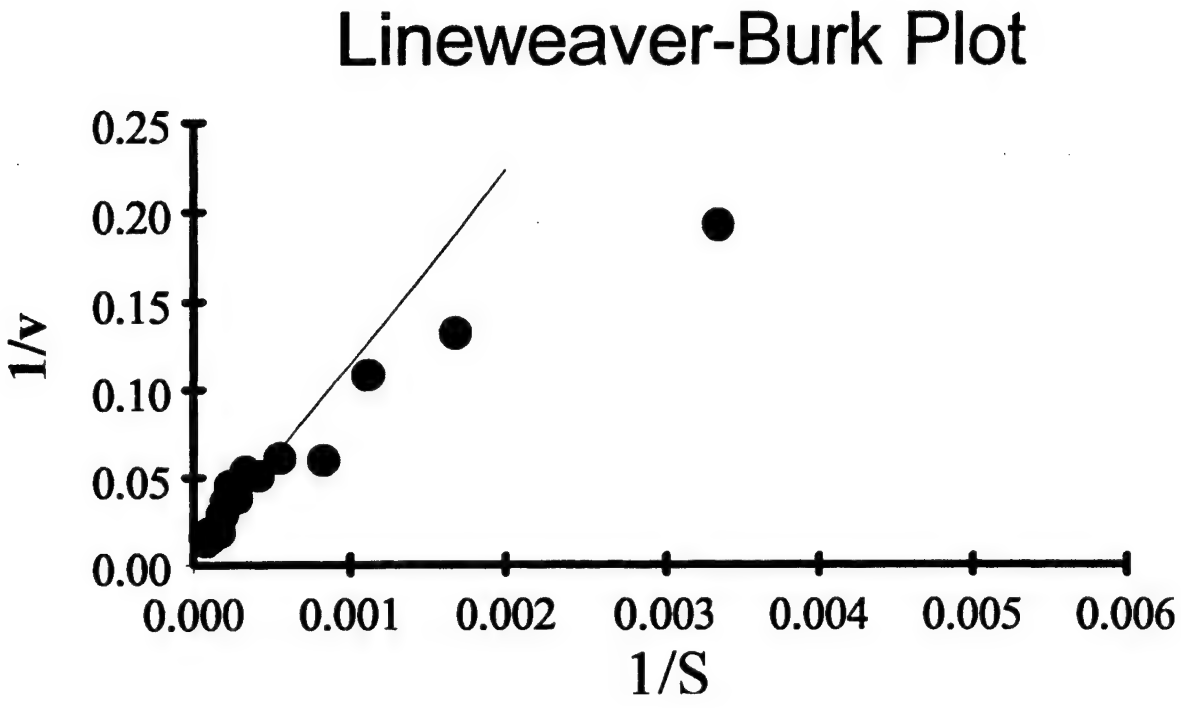
Saturation Plot: I106T/F132A/H254G/H257W on GB



Lineweaver-Burk Plot: I106T/F132A/H254G/H257W on GB



G. Raw Data: H254G/H257R on GB

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Date: Tuesday April 23, 2002 2:51 pm
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Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= 1.778 Akaike Criterion= 110.257
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= 4.2418

Km:= 484.935 +/- 216.6772 (p=2.458E-02)
Vmax:= 187.3679 +/- 16.9577 (p=3.376E-07)

Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.823	1.000

==========

Velocity, Dose, Inhibitor
64.6447,300,0
110.615,600,0
136.66,900,0
147.382,1200,0
141.917,1800,0
151.073,2400,0
110.773,3000,0
147.189,3600,0
133.817,4200,0
195.494,4800,0
199.642,5400,0
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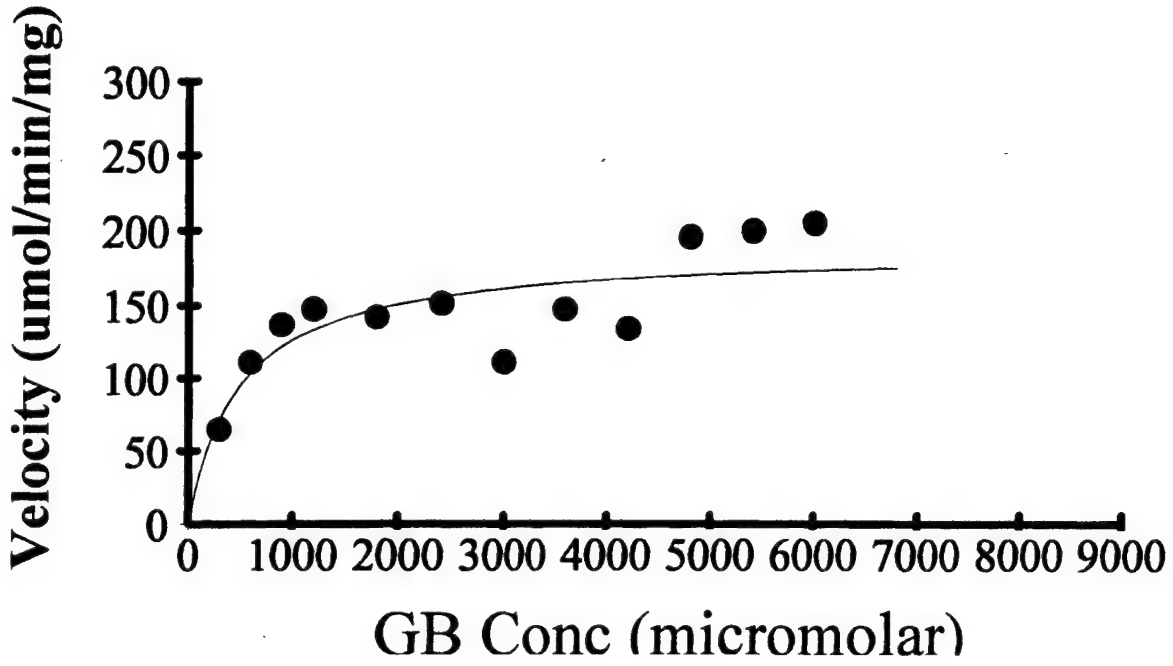
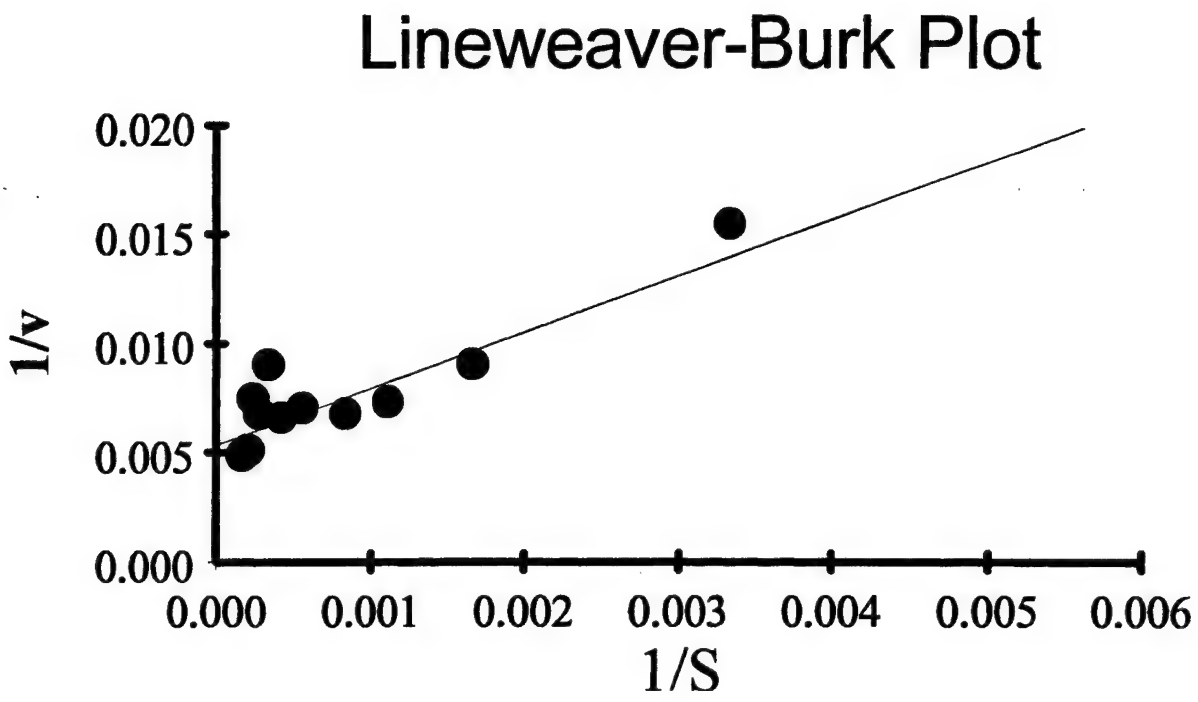
Saturation Plot: H254G/H257R on GB



Lineweaver-Burk Plot: H254G/H257R on GB



H. Raw Data: H254G/H259W/L303T on GB

Begin Results: C:\EZFIT5\HHL-GB.RAW

==========

Date: Tuesday May 14, 2002 9:00 am
Filename:
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= .739 Akaike Criterion= 15.153
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= 1.1823

Km:= 423.2063 +/- 162.2004 (p=2.386E-02)
Vmax:= 10.868 +/- 0.9653 (p=4.830E-05)

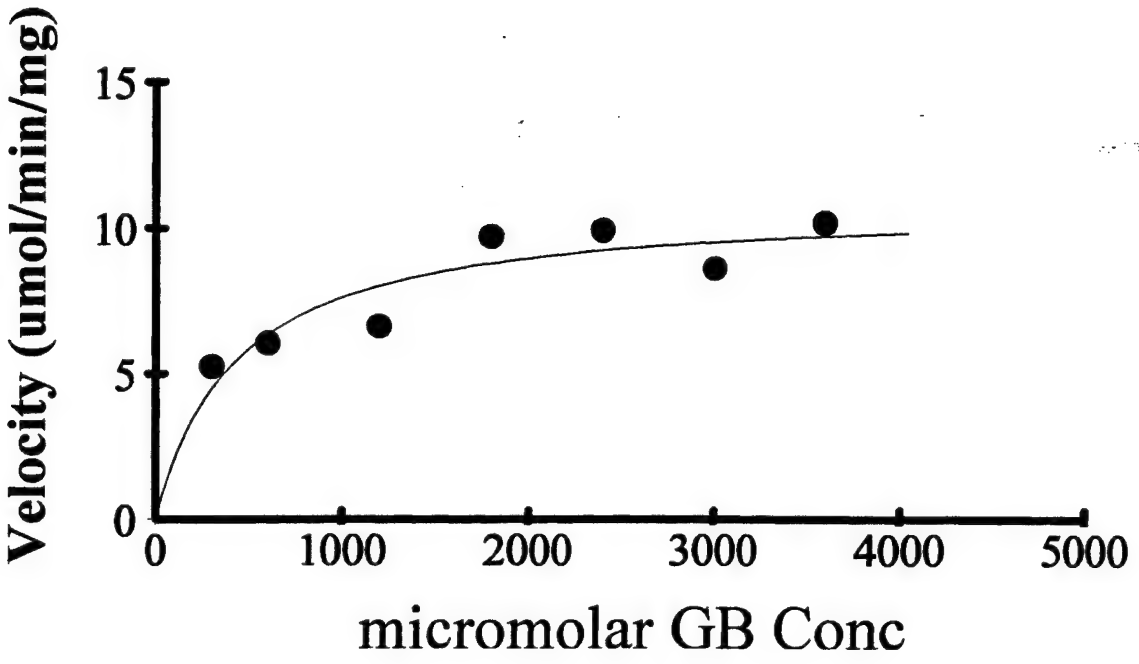
Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.858	1.000

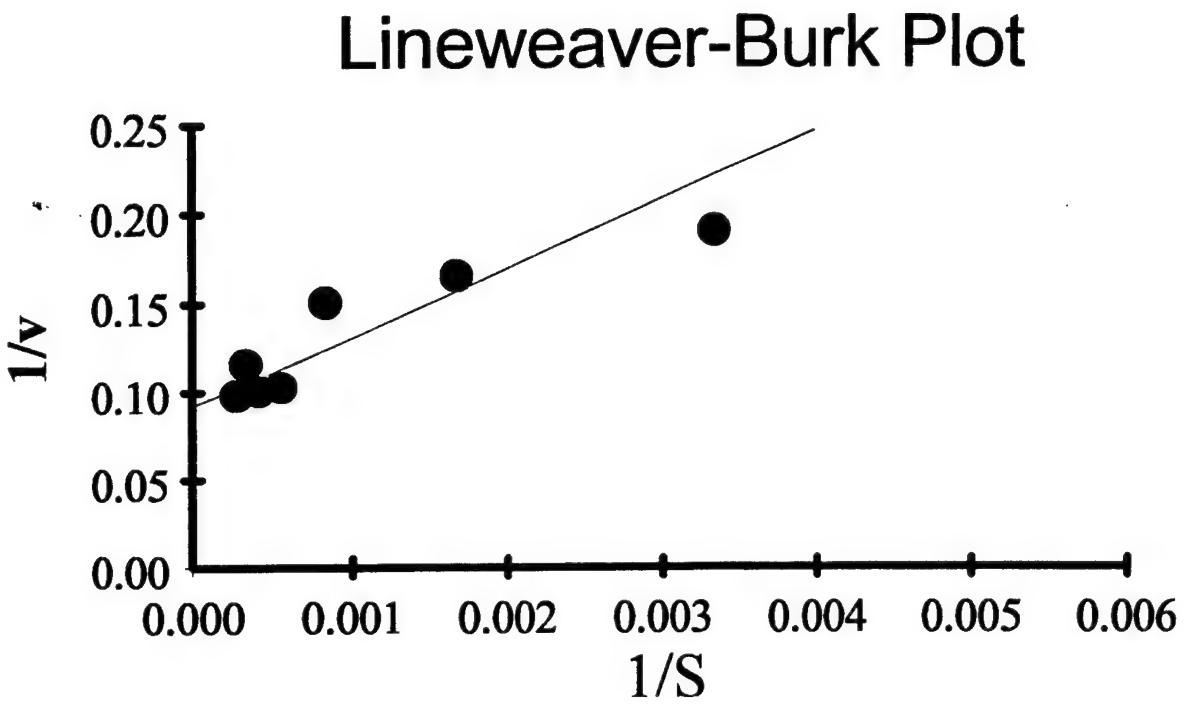
==========

Velocity, Dose, Inhibitor
5.24707,300,0
6.04064,600,0
6.63667,1200,0
9.70583,1800,0
9.92083,2400,0
8.61833,3000,0
10.1738,3600,0
End Results: C:\EZFIT5\HHL-GB.RAW

Saturation Plot: H254G/H259W/L303T on GB



Lineweaver-Burk Plot: H254G/H259W/L303T on GB



I. Raw Data: Wild-type on GD

==========

Date: Thursday January 31, 2002 11:03 am
Filename: C:\EZFIT5\WT-GD2.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -.185 Akaike Criterion= 71.965
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .5775

Km:= 7817.608 +/- 2774.374 (p=1.006E-02)
Vmax:= 129.7698 +/- 23.3065 (p=1.741E-04)

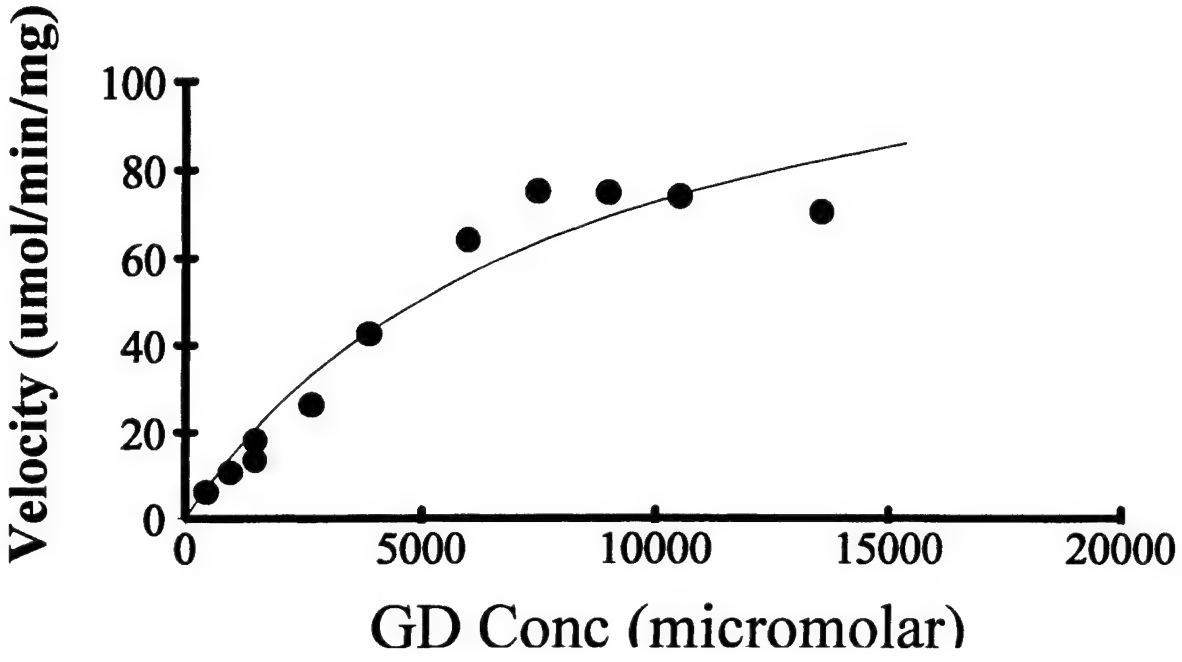
Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.970	1.000

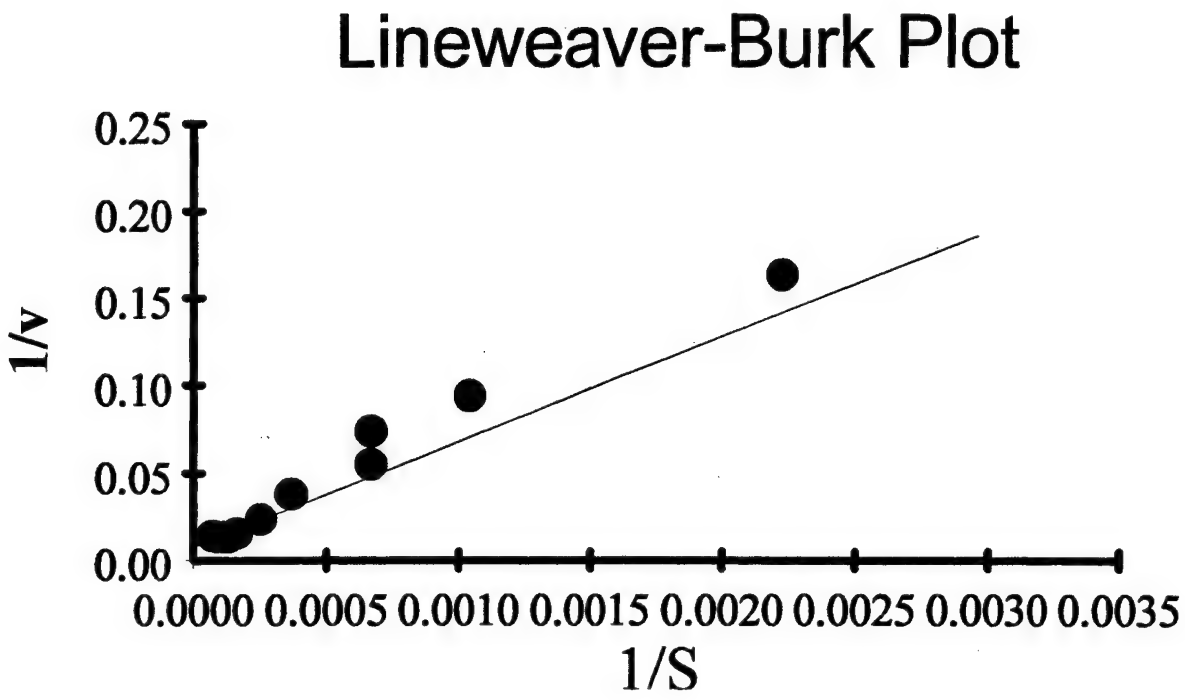
==========

Velocity, Dose, Inhibitor
6.125674,450,0
10.664,960,0
13.52178,1500,0
18.10904,1500,0
26.36607,2700,0
42.60667,3900,0
64.00711,6000,0
75.05704,7500,0
74.73007,9000,0
73.90356,10500,0
70.39511,13500,0
End Results: C:\EZFIT5\WT-GD2.RAW

Saturation Plot: Wild-type on GD



Lineweaver-Burk Plot: Wild-type on GD



J. Raw Data: I106A/S308A/H257Y PTE on GD

==========

Date: Thursday January 31, 2002 11:20 am
Filename: C:\EZFIT5\ISH-GD.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -.412 Akaike Criterion= 20.331
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .4016

Km:= 7763.558 +/- 3834.294 (p=4.465E-02)
Vmax:= 22.3309 +/- 6.2461 (p=5.855E-03)

Parameter Correlation Coefficient Matrix:

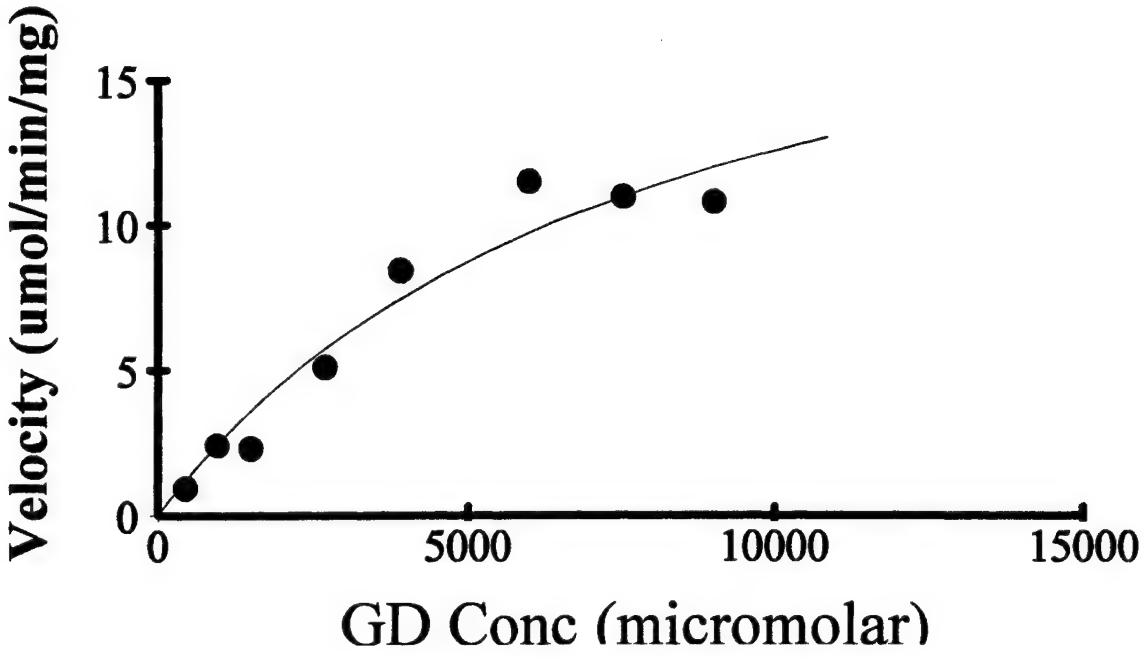
	Km	Vmax
1	1.000	
2	0.982	1.000

==========

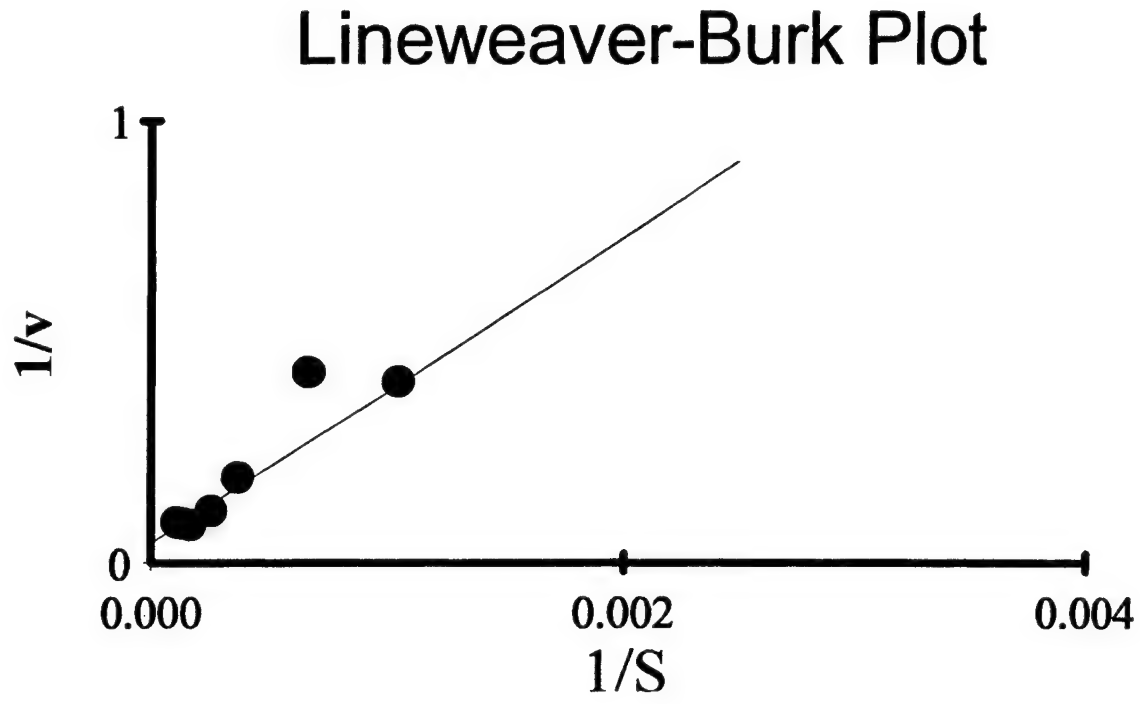
Velocity, Dose, Inhibitor
0.910168,450,0
2.404258,960,0
2.284403,1500,0
5.095318,2700,0
8.40463,3900,0

11.4768,6000,0
10.94962,7500,0
10.77747,9000,0
End Results: C:\EZFIT5\ISH-GD.RAW

Saturation Plot: I106A/S308A/H257Y PTE on GD



Saturation Plot: I106A/S308A/H257Y PTE on GD



K. Raw Data: I106A/F132A/H257Y on GD

==========

Date: Thursday January 31, 2002 9:38 am
Filename: C:\EZFIT5\ISH-GD.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -2.649 Akaike Criterion= -2.376
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0363

Km:= 6748.097 +/- 2041.334 (p=1.489E-02)
Vmax:= 23.2026 +/- 4.9204 (p=4.601E-03)

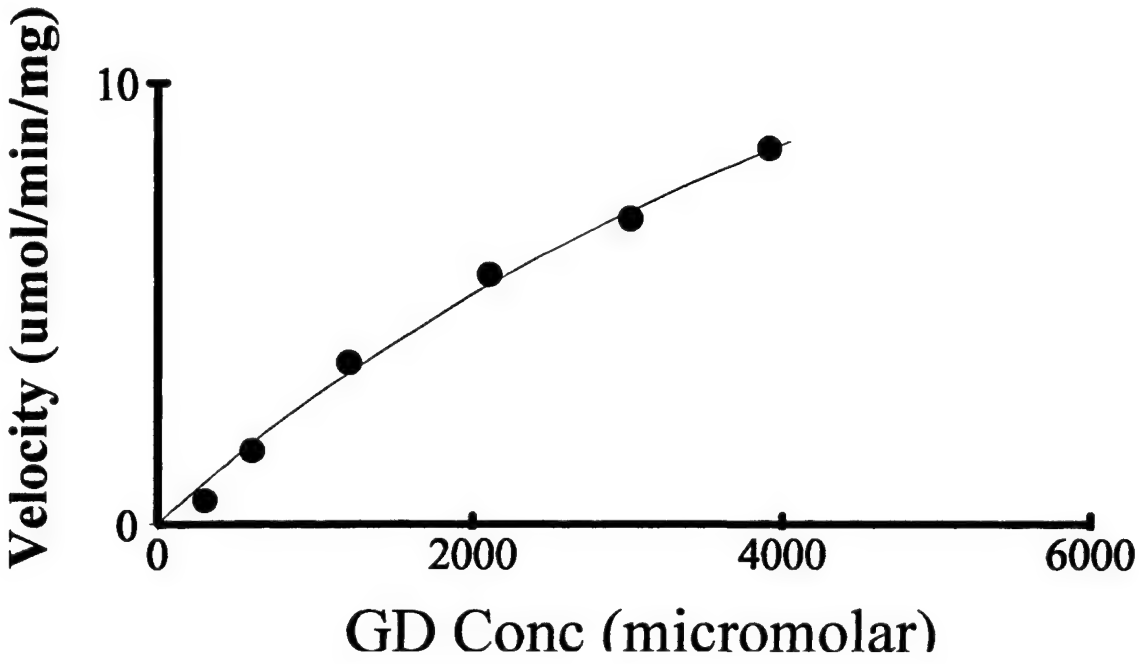
Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.994	1.000

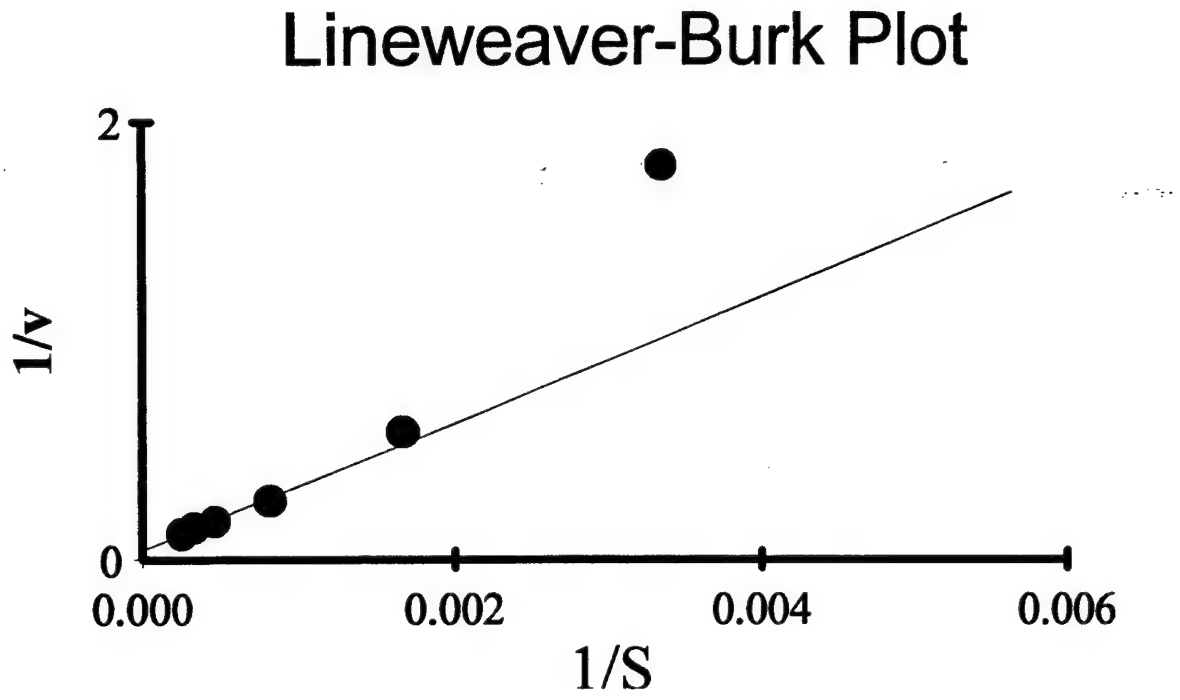
==========

Velocity, Dose, Inhibitor
.5557986,300,0
1.7096111,600,0
3.7210069,1200,0
5.7172068,2100,0
6.9594907,3000,0
8.5158179,3900,0
End Results: C:\EZFIT5\ISH-GD.RAW

Saturation Plot: I106A/F132A/H257Y on GD



Lineweaver-Burk Plot: I106A/F132A/H257Y on GD



L. Raw Data: G60A on GD

==========

Date: Thursday January 31, 2002 2:35 pm
Filename: C:\EZFIT5\ISH-GD.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -.285 Akaike Criterion= 77.73
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .5529

Km:= 2756.096 +/- 627.628 (p=5.394E-04)
Vmax:= 89.7141 +/- 6.9453 (p=2.573E-08)

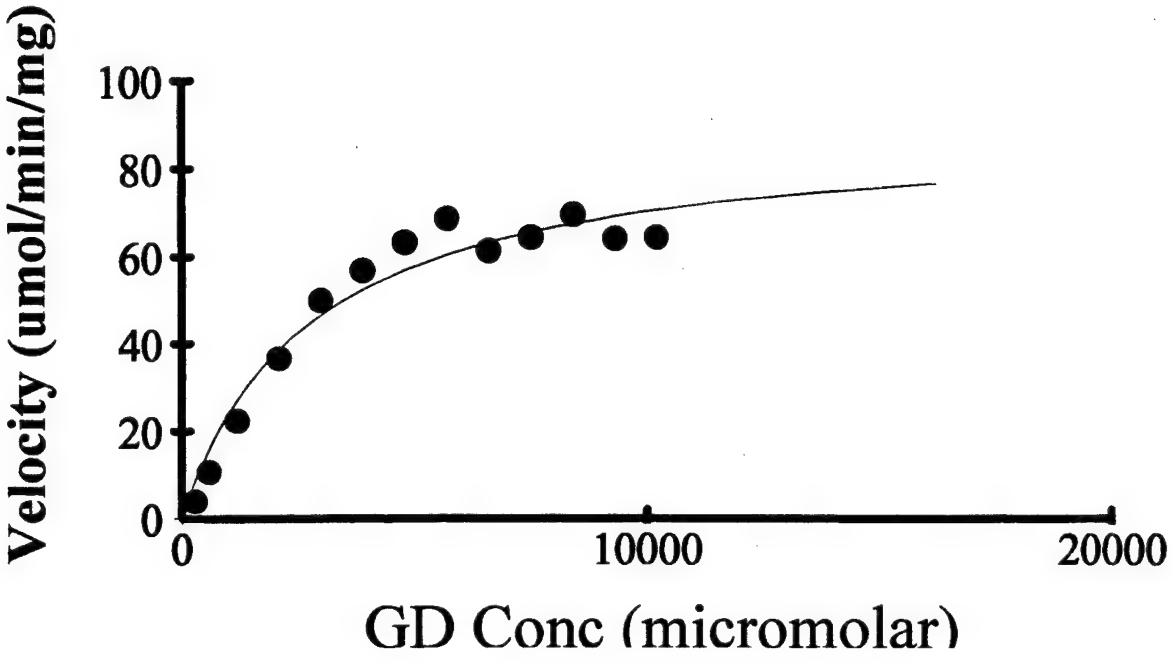
Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.939	1.000

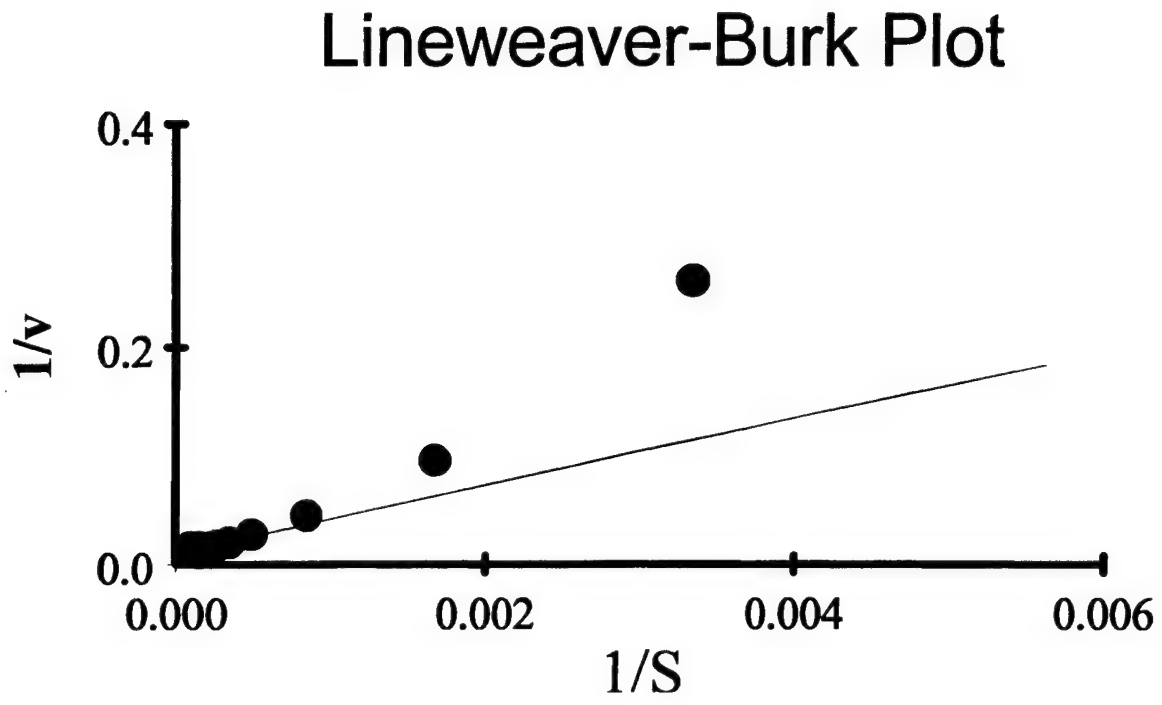
==========

Velocity, Dose, Inhibitor
3.8421437,300,0
10.539544,600,0
22.311261,1200,0
36.608305,2100,0
49.977553,3000,0
56.793865,3900,0
63.224841,4800,0
68.558923,5700,0
61.360269,6600,0
64.347924,7500,0
69.5159,8400,0
64.080808,9300,0
64.393565,10200,0
End Results: C:\EZFIT5\ISH-GD.RAW

Saturation Plot: G60A on GD



Lineweaver-Burk Plot: G60A on GD



M. Raw Data: I106T/F132A/H254G/H257W on GD

Begin Results: C:\EZFIT5\IFHH-GD.RAW

==========

Date: Wednesday April 17, 2002 8:44 am
Filename: C:\EZFIT5\IFHH-GD.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -2.104 Akaike Criterion= -8.244
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .0689

Km:= 2927.338 +/- 576.0198 (p=1.914E-03)
Vmax:= 9.4712 +/- 1.0218 (p=1.228E-04)

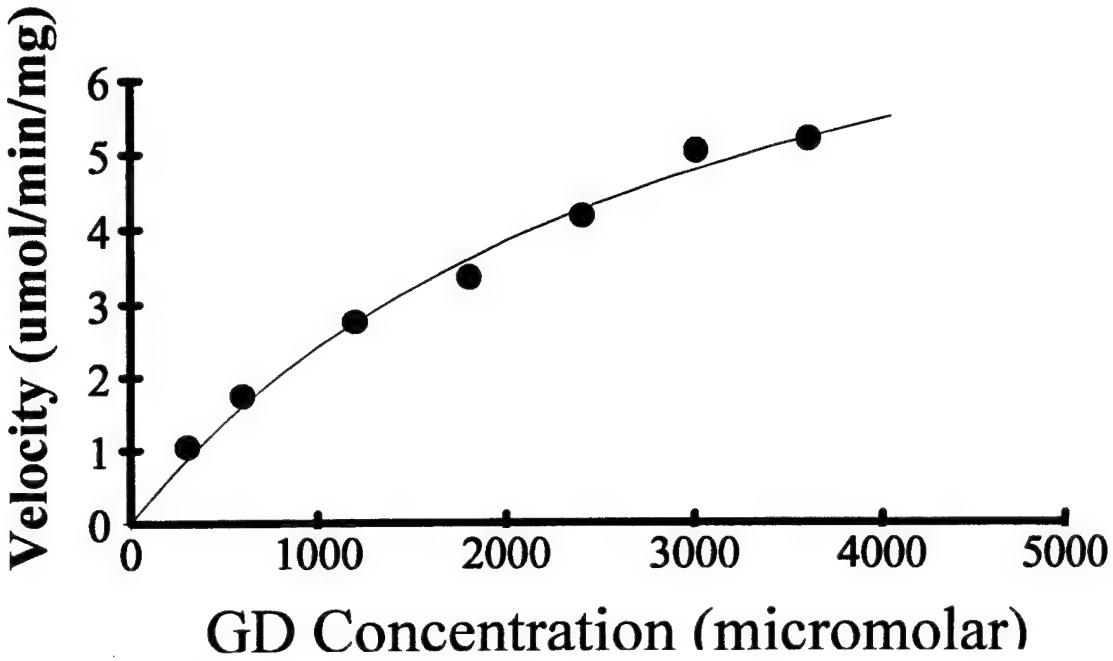
Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.984	1.000

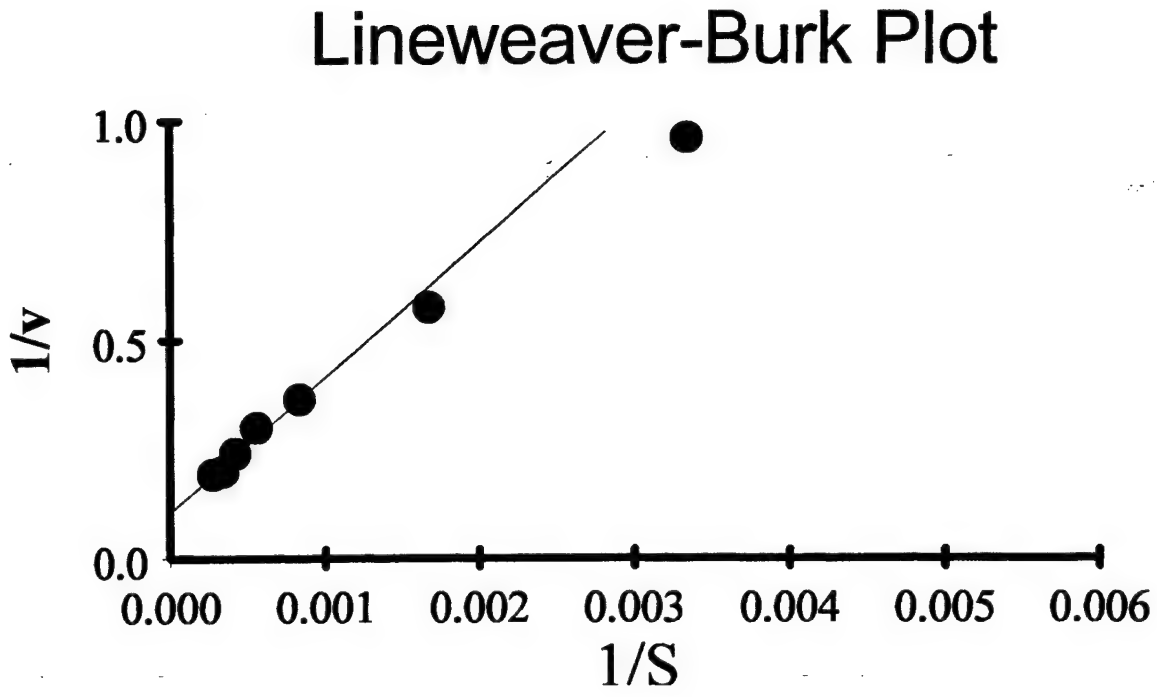
==========

Velocity, Dose, Inhibitor
1.04249,300,0
1.74135,600,0
2.75198,1200,0
3.35587,1800,0
4.16865,2400,0
5.03286,3000,0
5.19056,3600,0
End Results: C:\EZFIT5\IFHH-GD.RAW

Saturation Plot: I106T/F132A/H254G/H257W on GD



Lineweaver-Burk Plot: I106T/F132A/H254G/H257W on GD



N. Raw Data: H254G/H257R on GD

Begin Results: C:\EZFIT5\HH-GD.RAW

==========

Date: Wednesday April 17, 2002 9:01 am
Filename: C:\EZFIT5\HH-GD.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit= -.539 Akaike Criterion= 41.799
% Outliers= -Turned Off- Weight Factor= 1
RUNS test of Residuals= Pass p=0.05 1 - r^2= .3911

Km:= 3897.164 +/- 1271.771 (p=7.740E-03)
Vmax:= 56.4234 +/- 9.6835 (p=1.966E-04)

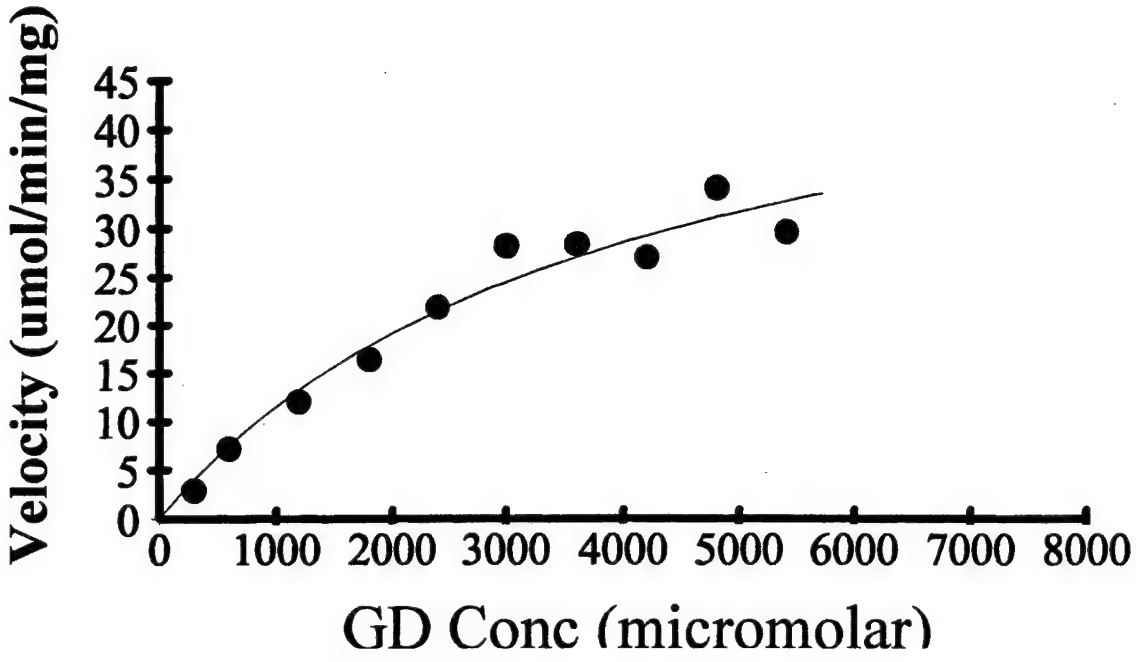
Parameter Correlation Coefficient Matrix:

	Km	Vmax
1	1.000	
2	0.982	1.000

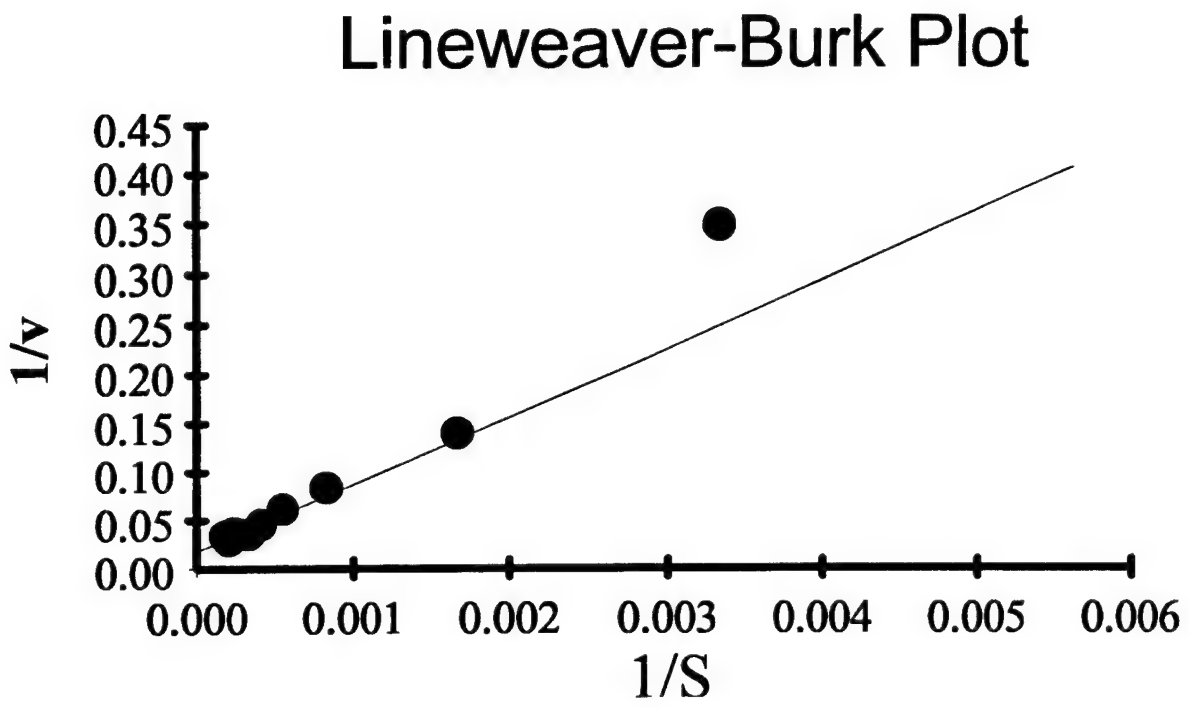
==========

Velocity, Dose, Inhibitor
2.87172,300,0
7.15829,600,0
12.0073,1200,0
16.364,1800,0
21.843,2400,0
28.178,3000,0
28.3348,3600,0
27.0154,4200,0
34.0515,4800,0
29.588,5400,0
End Results: C:\EZFIT5\HH-GD.RAW

Saturation Plot: H254G/H257R on GD



Lineweaver-Burk Plot: H254G/H257R on GD



O. Raw Data: H254G/H259W/L303T on GD

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==========================================================
Date: Tuesday  May 14, 2002      9:35 am
Filename: C:\EZFIT5\HHL-GD.RAW
Kinetic Model: MICHAELIS-MENTEN
Goodness-of-Fit=  .636              Akaike Criterion= -.592
% Outliers= -Turned Off-             Weight Factor= 1
RUNS test of Residuals= Pass p=0.05    1 - r^2=  .9694

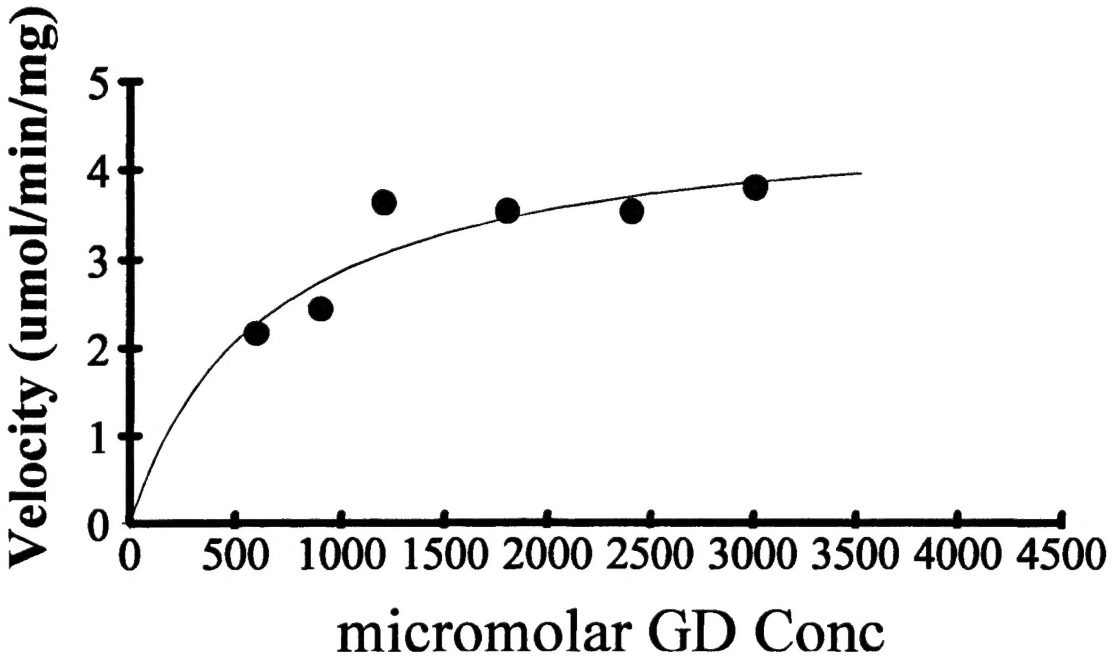
Km:= 622.2395    +/- 255.3245  (p=3.572E-02)
Vmax:= 4.6595    +/- 0.5673  (p=5.986E-04)

 Parameter Correlation Coefficient Matrix:
  Km         Vmax
1  1.000
2  0.935       1.000

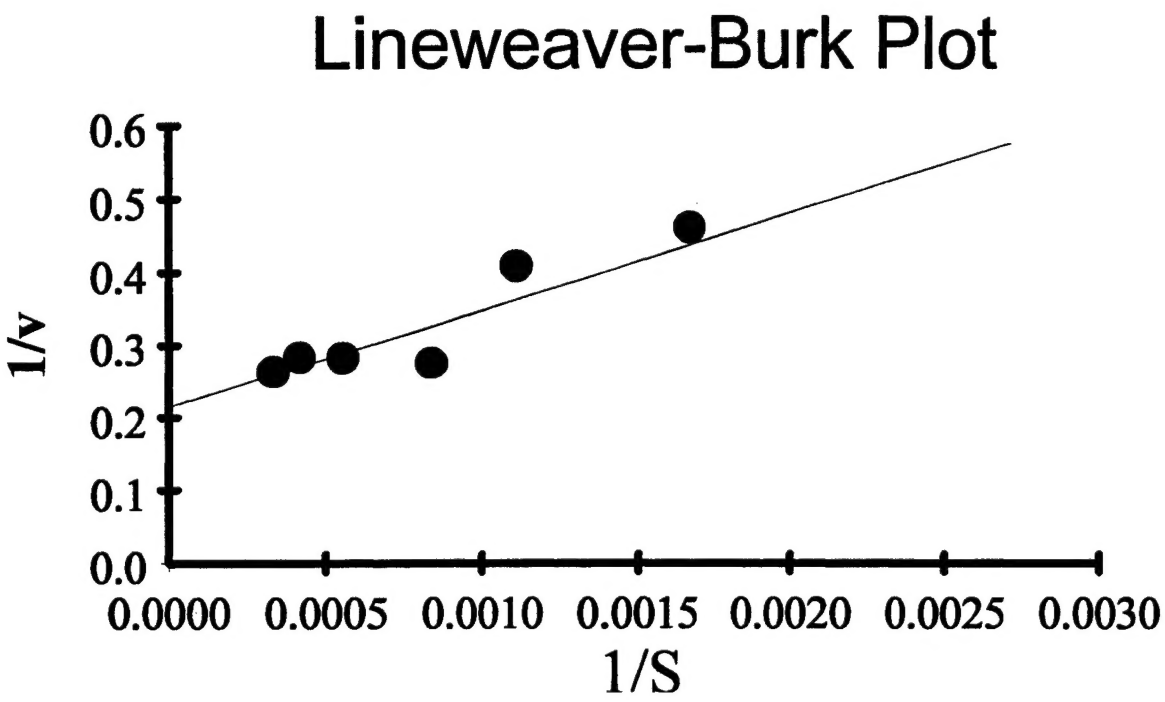
==========================================================

Velocity, Dose, Inhibitor
2.16852,600,0
2.44548,900,0
3.63237,1200,0
3.53489,1800,0
3.53156,2400,0
3.79867,3000,0
End Results: C:\EZFIT5\HHL-GD.RAW
```

Saturation Plot: H254G/H259W/L303T on GD



Lineweaver-Burk Plot: H254G/H259W/L303T on GD



P. Summary tables of kinetics of wild-type and mutant PTE enzymes on GB and GD.

Table 1. GB kinetics summary. Activity of wild-type and mutant strains on GB. k_{cat} calculations are based on an observed dimeric molecular weight of 70,000 daltons for the OPH enzyme.

Genotype	Km (μM)	Vmax (μmol/min/mg)	k_{cat} (sec^{-1})	K_{cat}/Km (μM^{-1} sec^{-1})
Wild-type (WT)	1,040 +/- 84	1,300 +/- 28	1517 +/- 33	1.459 +/- 0.15
I106A/S308A/H257Y (ISH)	1,778 +/- 540	833.2 +/- 110	972.1+/- 120	0.5468 +/- 0.24
I106A/F132A/H257Y(IFH)	1,125 +/- 310	318.8 +/- 30	372.0 +/- 35	0.3307 +/- 0.12
Co-RI106A/H257Y (Co-R-IH)	1,494 +/- 330	1,098 +/- 100	1,281 +/- 120	0.8574 +/- 0.27
G60A	433.3 +/- 150	186.9 +/- 15	218.1 +/- 17	0.5033 +/- 0.21
I106T/F132A/H254G/H257W (IFHH)	20,280 +/- 8,700	185.2 +/- 56	216.1 +/- 65	0.01066 +/- 0.0078
H254G/H257R (HH)	484.9 +/- 220	187.4 +/- 17	218.6 +/- 20	0.4508 +/- 0.24
H254G/H259W/L303T	423.2 +/- 162	10.87 +/- 0.965	12.68 +/- 11.3	0.02996 +/- 0.0014

Table 2. GD kinetics summary. Activity of wild-type and mutant strains on GD. k_{cat} calculations are based on an observed dimeric molecular weight of 70,000 daltons for the OPH enzyme.

Genotype	Km (μM)	Vmax (μmol/min/mg)	k_{cat} (sec^{-1})	K_{cat}/Km (μM^{-1} sec^{-1})
Wild-type	7817 +/- 28	129.8 +/- 23	151.4 +/- 27	0.01937 +/- 0.010
I106A/S308A/H257Y	7764 +/- 38	22.33 +/- 6.3	26.05 +/- 7.3	0.003355 +/- 0.0026
I106A/F132A/H257Y	6748 +/- 20	23.20 +/- 4.9	27.07 +/- 5.7	0.004012 +/- .0021
Co-RI106A/H257Y	ND	ND	ND	ND
G60A	2756 +/- 630	89.71 +/- 6.9	104.7 +/- 8.1	0.0380 +/- .010
I106T/F132A/H254G/H257W	2927 +/- 580	9.471 +/- 1.0	11.05 +/- 1.2	0.003908 +/- 0.0012
H254G/H257R	3897 +/- 1300	56.42 +/- 10	65.83 +/- 11	0.01689 +/- .0084
H254G/H259W/L303T	622.2 +/- 260	4.6595 +/- 0.57	5.436 +/- 0.66	0.008736 +/- 0.0046